The American Journal of Medicine

September 1950

The American Journal of Medicine

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Packaging: MICROPELLETS PROGYNON: Multiple dose vials of 10 cc. containing 0.25 and 1.0 mg. estradiol (3000 and 12,000 R.U.) per cc. Boxes of 1 and 6 vials.

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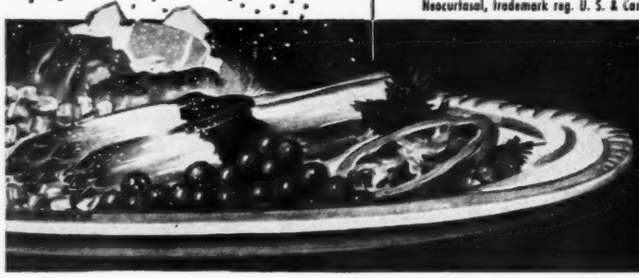
Sodium restriction is an essential part of the modern management of cardiovascular failure. But, without seasoning, low sodium diets are difficult to endure. Neocurtasal, completely sodium free salt, palatably seasons all foods. Neocurtasal looks and is used like ordinary table salt. Available in convenient 2 oz. shakers and 8 oz. bottles. Constituents: Potassium chloride, ammonium chloride, potassium formate, calcium formate, magnesium citrate and starch. Potassium content 36%; chloride 39.3%; calcium 0.3%; magnesium 0.2%.

Neocurtasal, trademark reg. U. S. & Canada

Write for pads of diet sheets.



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CONTENTS

The American Journal of Medicine

VOL. IX SEPTEMBER, 1950 NO. 3

Editorial

Clinical Studies

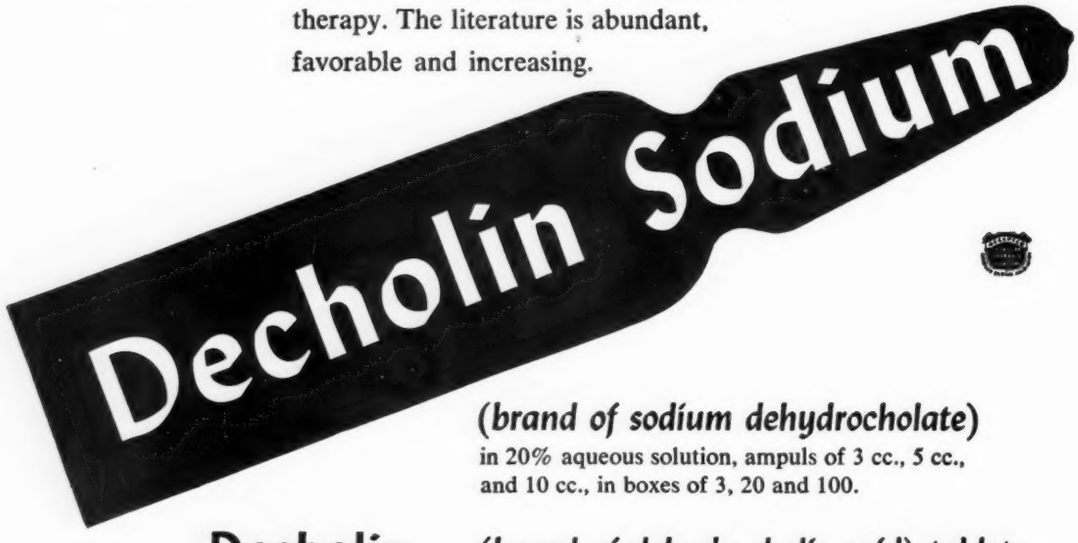
Contents continued on page 5

Major points of difference

point one — **Decholin Sodium** is a true *hydro*choleretic agent. Its administration in biliary tract therapy stimulates and increases hepatic output of thin, low viscosity, "tract-rinsing" bile.

point two — **Decholin Sodium** is a single, specific bile acid derivative—pure sodium dehydrocholate, gram for gram the most effective *hydro*choleretic available to medicine. Adequate dosage has been established and action is predictable.

point three — **Decholin Sodium** has become established as the clinical standard for *hydro*choleretic therapy. The literature is abundant, favorable and increasing.



(brand of sodium dehydrocholate)
in 20% aqueous solution, ampuls of 3 cc., 5 cc., and 10 cc., in boxes of 3, 20 and 100.

Decholin ***(brand of dehydrocholic acid) tablets***
in bottles of 25, 100, 500, 1000 and 5000.

Decholin and *Decholin Sodium*, reg. trademarks

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CONTENTS

The American Journal of Medicine

VOL. IX SEPTEMBER, 1950 NO. 3

Contents continued from page 3

Review

Seminars on Renal Physiology

Conference on Therapy

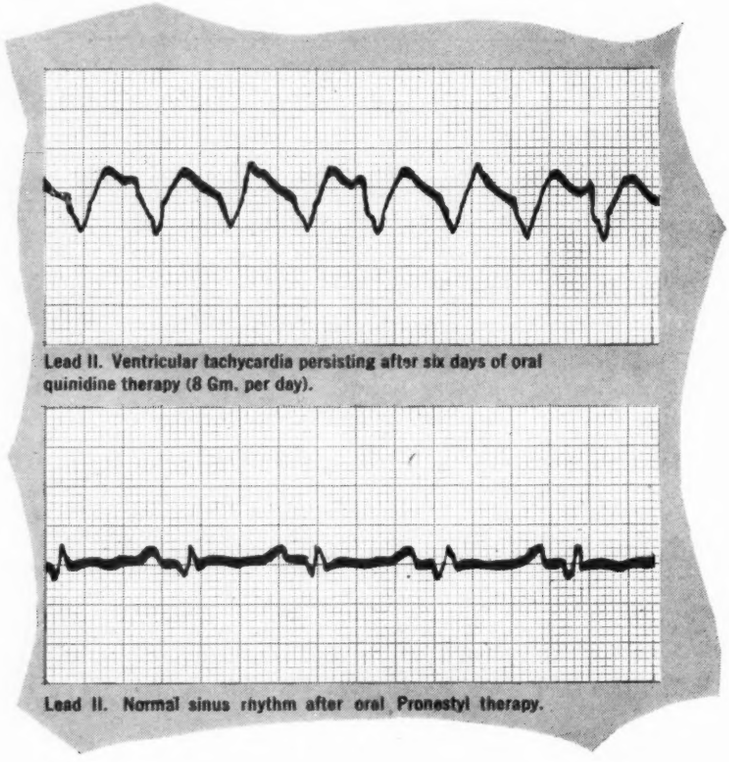
Contents continued on page 7

a new drug . . .

for the treatment of ventricular arrhythmias

PRONESTYL *Hydrochloride*

Squibb Procaine Amide Hydrochloride



Lead II. Ventricular tachycardia persisting after six days of oral quinidine therapy (8 Gm. per day).

Lead II. Normal sinus rhythm after oral Pronestyl therapy.

Oral administration of Pronestyl in doses of 3-6 grams per day, for periods of time varying from 2 days to 3 months, produced no toxic effects as evidenced by studies of blood count, urine, liver function, blood pressure, and electrocardiogram. Pronestyl may be given intravenously with relative safety.

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CONTENTS

The American Journal of Medicine

VOL. IX SEPTEMBER, 1950 NO. 3

Contents continued from page 5

Clinico-pathologic Conference

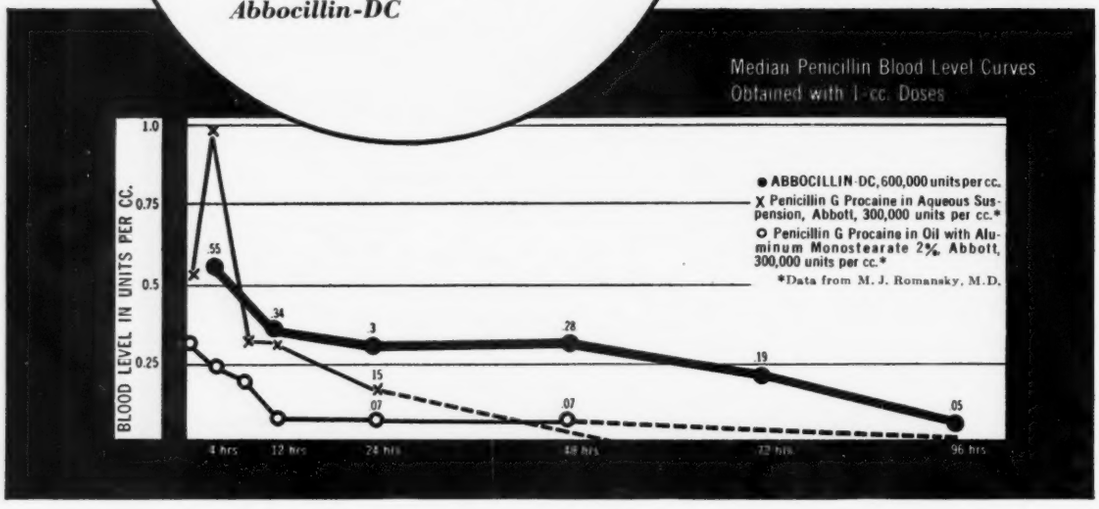
Special Feature

Case Reports

Compare the

sustained high blood levels

obtained with a single 1-cc. dose of 600,000-unit Abbocillin-DC



This is a truly repository preparation—600,000 units (double concentration) of penicillin G procaine in aqueous suspension. Single 1-cc. doses of ABBOCILLIN-DC have consistently afforded prolonged high blood levels similar to the median curve shown above.

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Try it the next time repository penicillin is indicated. Abbott

NOTE THE NAME... **Abbocillin-DC**

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*T. M. Reg. Becton, Dickinson & Co.

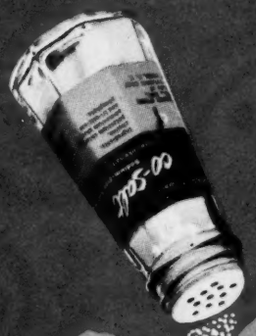
now

patients on low-sodium diets

will enjoy their meals again

in
CONGESTIVE HEART FAILURE
TOXEMIAS OF PREGNANCY
HYPERTENSION

CO-SALT tastes and seasons food so much like sodium chloride that patients on low-sodium diets will hardly miss salt. They will cooperate more fully in adhering to your diet, be better nourished, keep intake of edema-causing sodium at a minimum.



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the new and different sodium-free salt substitute

- **tastes like salt**
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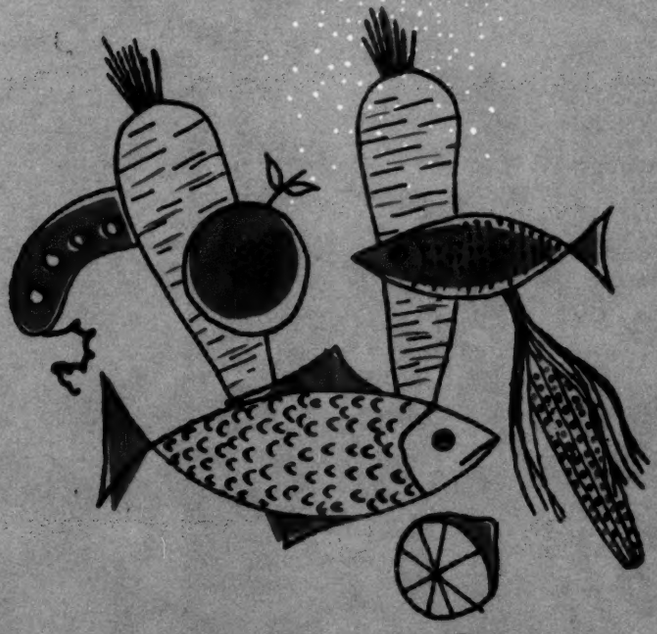
- **sprinkles like salt**

Accepted for advertising in the Journal of the American Medical Association.



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CO-SALT contains: Choline, Potassium Chloride, Ammonium Chloride and Tri-Calcium Phosphate.

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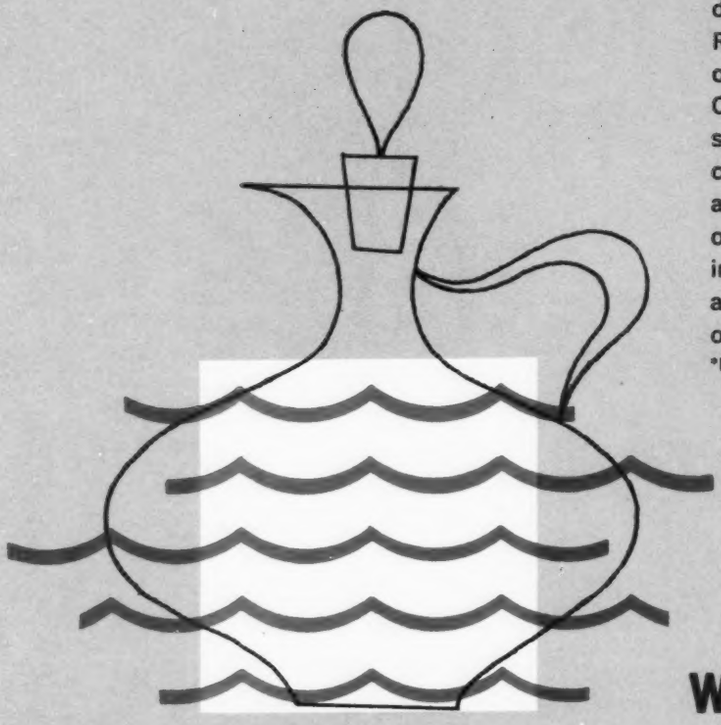
*U. S. Patent No. 2,417,299.



water

gets the oil there

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Provides per gram (approx. 1 cc.) 50,000 U. S. P. units of natural vitamin A in aqueous solution.

Particularly suggested in patients with dysfunctions of the liver, pancreas, and biliary tract which interfere with utilization of fats; in celiac disease and certain other diarrheal states.

1. Lewis, J. M., Cohlan, S. Q., and Messina, A.: Pediatrics 5:425, 1950.
2. Lewis, J. M., and Cohlan, S. Q.: Med. Clinics North Amer., March, 1950.

Send for samples.

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the
protection
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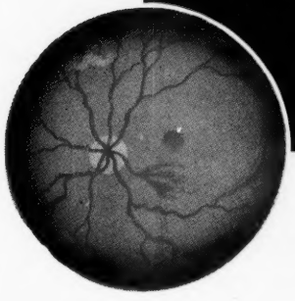
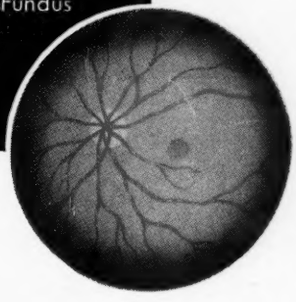
complicating
hazard
—bottles
of
100
tablets

Ocular Fundus in Degenerative Vascular Disease—Hypertension, Diabetes, Arteriosclerosis—note tortuous blood vessels, areas of exudation, hemorrhagic areas.



Normal Ocular Fundus



[1]In keeping with newer clinical findings, the rutin content of RUTAMINAL has been increased to 60 mg. per tablet (three times the former rutin content) at no increase in cost to the patient.

***RUTAMINAL is the trademark of Schenley Laboratories, Inc. and designates exclusively its brand of tablets containing rutin, aminophylline, and phenobarbital.**

schenley laboratories, inc., **350 fifth ave., new york 1, n. y.**

a new type antihistaminic

'PERAZIL' BRAND

CHLORCYCLIZINE HYDROCHLORIDE

℞ *on prescription only*

New because its distinctive chemical component is a piperazine ring instead of the ethylenediamine group on which most antihistaminic compounds have so far been based; greater specificity of action is the result.

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"The percentage and severity of side reactions was very low. Due to the longer duration of action of 'Perazil', less frequent administration of tablets was necessary".

Cullick, L. and Ogden, H. D.: J. South Med. Assn. 43: *No. 7, July 1950*

INDICATIONS:	Hay fever, vasomotor rhinitis, urticaria, allergic dermatitis and pollen asthma.
DOSAGE:	50 mg. (one product) once or twice daily with water; may be increased if required in severe cases.
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Orange flavor tastes delicious straight on the tongue or mixed in food. Non-allergenic, non-alcoholic Vitamin A ester used—no fishy taste or odor. No alcohol.

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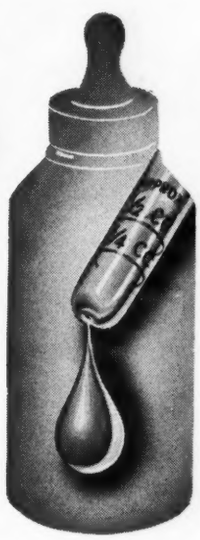
Each cc. of Ol-Vitum Drops contains:

Vitamin A ester	10,000 USP Units
Vitamin D	2,000 USP Units
Natural mixed tocopherols*	3 mg.
Thiamine hydrochloride	3 mg.
Riboflavin	0.8 mg.
Niacinamide	15 mg.
Pyridoxine hydrochloride	1.6 mg.
Ascorbic acid	100 mg.

No refrigeration necessary. No expiration dating required.



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EFFECTIVENESS

Infants receiving Vitamins A, C and D plus the B complex showed weight increases 20% to 50% greater than those receiving Vitamins A, D and C alone.[1]

In Bottles of 15 and 30 cc. with especially calibrated dropper. Dosage: ¼ to 1 cc. according to age.

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DROPS

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OL-VITUM® CAPSULES are also available

International Vitamin Division IVES-CAMERON COMPANY, INC., New York 16, N. Y.

1. Kasdon, S. C., and Cornell, E. L.: Am. J. Obstet. & Gynec. 56:853 (Nov.) 1948.

*Equivalent (by biological assay) to 1.5 mg. d, Alpha Tocopherol

Aureo-mycin

AUREOMYCIN
CRYSTALLINE

for complications following Acute Infections in Childhood

Now is the season for children to enter upon their scholastic labors, and in most communities to receive either primary, or booster, immunization against several of the common childhood infections. Reliance must be placed upon antibiotics to control the secondary invaders which may follow these infections. Pediatricians are increasingly turning to aureomycin for this purpose, because of its wide range of activity against the common Gram-positive and Gram-negative organisms.

Aureomycin is also indicated for the control of the following infections:

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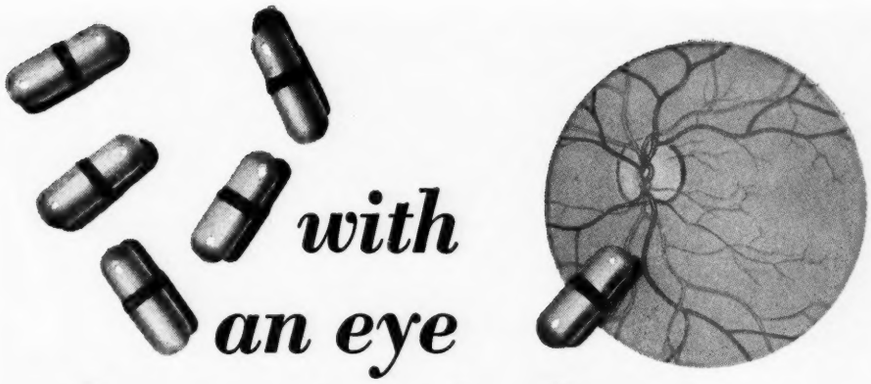
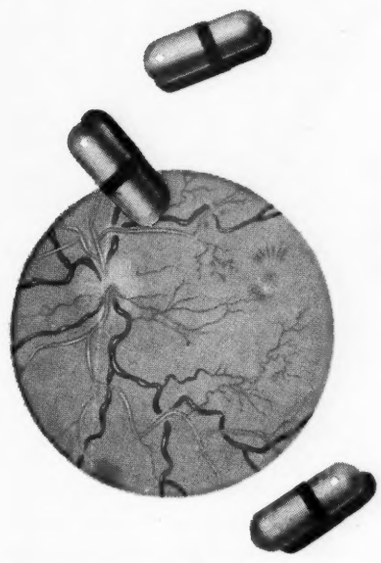
Capsules: Bottles of 25, 50 mg. each capsule. Bottles of 16, 250 mg. each capsule.
Ophthalmic: Vials of 25 mg. with dropper; solution prepared by adding 5 cc. of distilled water.

LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY 30 Rockefeller Plaza, New York 20, N. Y.



with an eye to the patient's future

To safeguard hypertensive, diabetic and certain other patients, RUCON KAPSEALS afford strategic and safe prophylaxis against capillary bleeding. The ever-present threat of vascular accident is minimized by combatting increased capillary fragility.



RUCON*

KAPSEALS®

RUCON KAPSEALS give three-fold protection to patients with increased capillary fragility associated with hypertension, diabetes mellitus, pulmonary hemorrhage, retinal hemorrhage, hereditary hemorrhagic telangiectasia, thiocyanate therapy, ascorbic acid deficiency and drug sensitivity. Rutin increases capillary strength, vitamin C maintains intercellular substance and integrity of capillary endothelium, and calcium aids the coagulation process.

Each RUCON Kapseal contains:

Rutin	100 mg.
Vitamin C (Ascorbic Acid)	100 mg.
Dicalcium Phosphate Anhydrous	400 mg.

DOSAGE: One RUCON Kapseal daily may be given initially, to be increased in accordance with therapeutic requirements. In some patients dosages of 300 mg. daily of rutin (3 RUCON Kapseals) may be required to secure adequate response. The Göthlin Petechial Index, determined prior to instituting therapy and repeated frequently during treatment, may be helpful as a guide to therapy.

RUCON Kapseals are supplied in bottles of 100.

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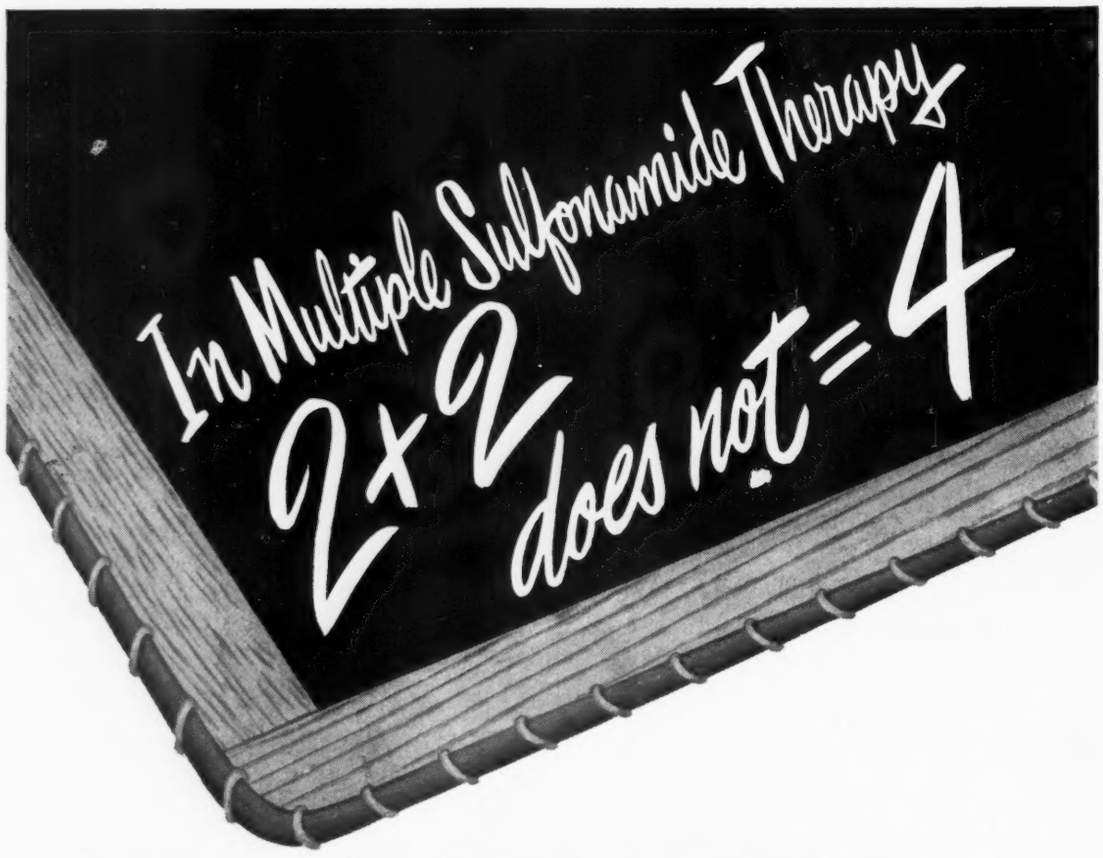

Therapeutic action is additive, but the total urinary solubility of two sulfonamides is significantly greater than that of either alone.

Aldiazol-M, a dual sulfonamide, contains both sulfadiazine and sulfamerazine in microcrystalline form, and the alkalizing salt, sodium citrate. Aldiazol-M increases the field of useful application of sulfonamide therapy because:

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Widely indicated. Aldiazol-M is indicated whenever the specific actions of sulfadiazine and sulfamerazine are required. It is valuable not only in adults, but also in the treatment of many infectious diseases in children.

Aldiazol-M is available on prescription through all pharmacies. Write for literature.

THE S. E. MASSENGILL COMPANY
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NEW YORK • SAN FRANCISCO • KANSAS CITY

SEMCO

Each teaspoonful (5 cc.) of Aldiazol-M provides:
Sulfadiazine (microcrystalline)... 0.25 Gm.
Sulfamerazine (microcrystalline)... 0.25 Gm.
Sodium Citrate..... 1.0 Gm.

Aldiazol-M

[illegible] alkaloid[illegible] fraction of Veratrum viride—biologically s[illegible] [illegible] for hyp[illegible] in mammals—a new active [illegible] [illegible] heretofore [illegible] treatment of hyperten[illegible]

An entirely new drug, [illegible] only the [illegible] alkaloids of [illegible] the irritant and inert [illegible] of the crude root. [illegible] biologically [illegible] is absolutely [illegible] action.

Individ[illegible] for ma[illegible] bene- fit. In the ma[illegible] verage d[illegible] to 5.0 mg. three or four times [illegible] in a clinically significant drop in [illegible] relaxation of the smaller arterioles. [illegible] for as long as the drug is given, for tolerance to [illegible] does not develop. Concomitantly, gratifying subjective improvement is discernible by the patient.

Veriloid has been found effective in malignant hypertension, severe essential hypertension, and in moderate elevation of the blood pressure. It is available on prescription through all pharmacies in slow dissolving tablets containing 1.0 mg. in bottles of 100 and 200.

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BIOLOGICALLY STANDARDIZED FOR HYPOTENSIVE POTENCY IN MAMMALS

now

peptomatic* digestional aid in single tablet form

By developing an entirely new type of enzymatic carrier, literally "a tablet within a tablet," Robins now makes available a triple-enzyme digestant—Entozyme. In *one small specially constructed tablet,* Entozyme "packs" pepsin, pancreatin and bile salts—in such a way that they are released only at the gastro-intestinal level of optimal activity. Thus Entozyme greatly simplifies and makes more effective the treatment of complex digestive disturbances of the gastro-intestinal tract. Clinical studies[1,2,3] have demonstrated the value of Entozyme in such conditions as chronic cholecystitis, chronic duodenal ulcer, acute and chronic pancreatitis and certain postoperative syndromes of the gastro-intestinal tract—in relieving nausea, belching, distention, anorexia, food intolerance, etc.

FORMULA: Each specially constructed tablet contains Pancreatin, U.S.P., 300 mg.; Pepsin, N.F., 250 mg.; Bile Salts, 150 mg.

DOSAGE: One or two tablets after each meal, or as directed by physician, without crushing or chewing.

AVAILABLE: Bottles of 25 and 100.

REFERENCES:

1. Kammandel, N. et al.: Awaiting publication.
2. McGavack, T. H. and Klotz, S. D.: Bull. Flower Fifth Ave. Hosp., 9:61, 1946.
3. Weissberg, J., McGavack, T. H. and Boyd, Linn J.: Am. J. Digest. Dis., 15:332, 1948.

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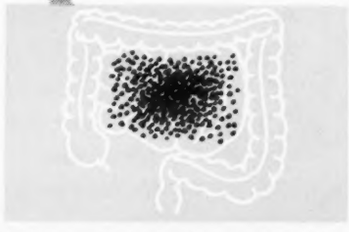
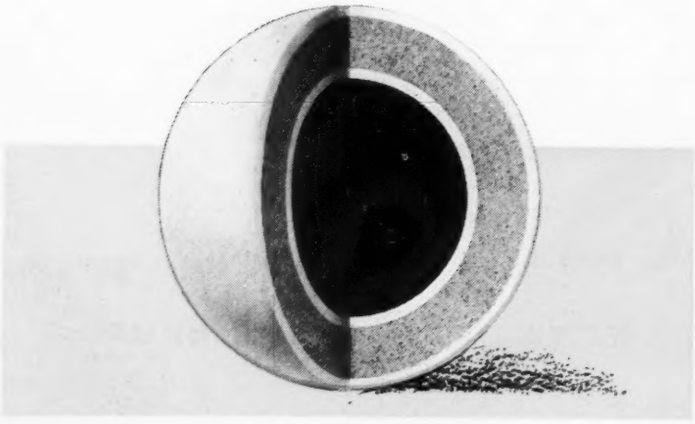
Ethical Pharmaceuticals of Merit since 1878

The "Peptomatic" Tablet

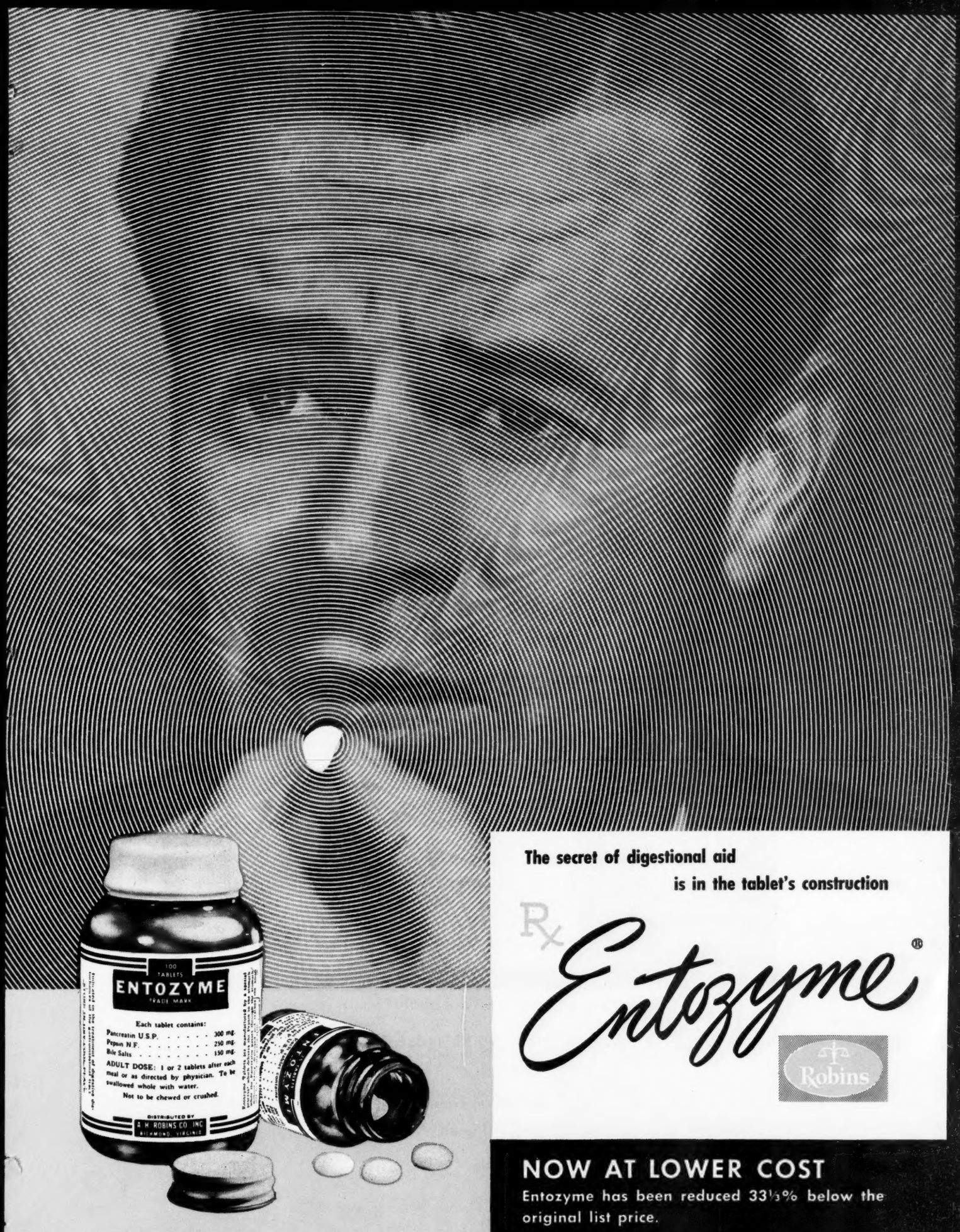
Releases pepsin in the stomach



Releases pancreatin and bile salts in the small intestine



***The "Peptomatic" Tablet—**
a coined word to describe the unique mechanical action of Entozyme Tablet.



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The development of the improved Biolac supplies a long-sought need in infant nutrition. To accomplish this, Borden scientists surveyed our present nutritional knowledge. They then tested more than 500 formulations. Having decided on the formula that would best supply the normal infant's nutritional requirements in their most assimilable form, a modern plant was constructed in 1949 so that the new formula could also benefit from the most up-to-date techniques and control in processing equipment. A Biolac formula that is both new and improved is thus made available.

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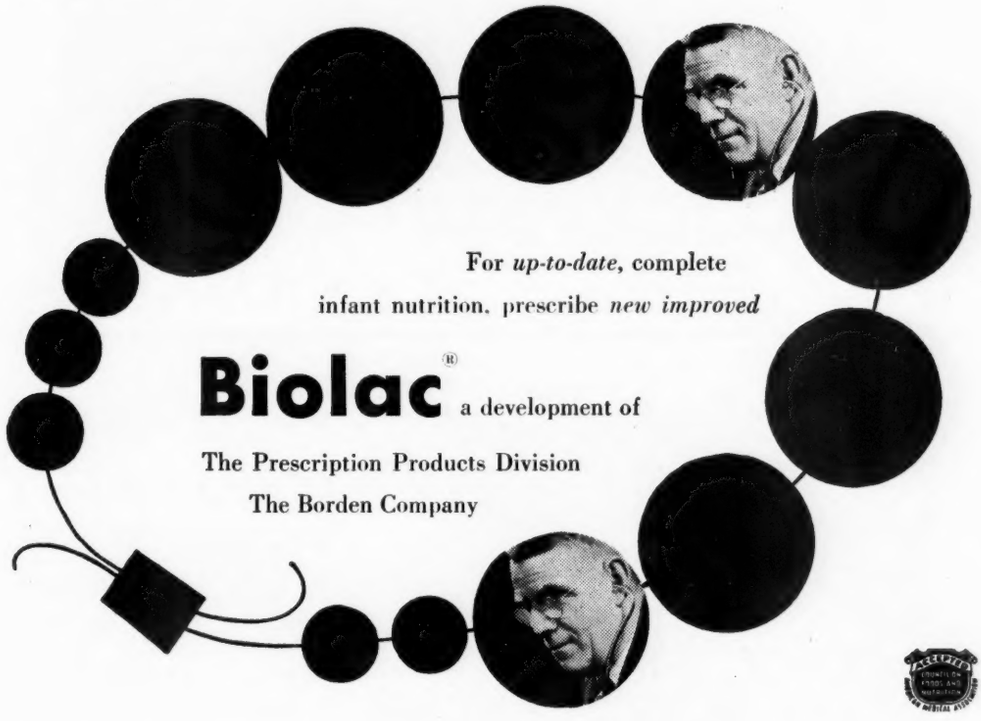
And yet, for all these advantages, Biolac costs no more.



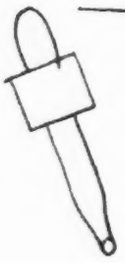
Ingredients: skim milk, dextrins-maltose-dextrose, lactose, coconut oil, destearinated beef fat, lecithin, sodium alginate, disodium phosphate, ferric citrate, vitamin B_1, concentrate of vitamins A and D from fish liver oils, and water.
Homogenized and sterilized.

Dilution: one fluid ounce to one and a half ounces of boiled water for each pound of body weight.

Biolac is available at drugstores in 13 fluid ounce tins.

The Borden Company, Prescription Products Division, 350 Madison Avenue, New York 17

Therapeutic penicillin dosage is EASY, PLEASANT with



DROP-CILLIN

50,000 UNITS* IN A DROPPERFUL

Available in 9cc. bottle containing 600,000 units.

DRAM-CILLIN

100,000 UNITS* IN A TEASPOONFUL

Available in 60cc. bottle containing 1,200,000 units.

Both with a palatable vanilla flavor.

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1. Weiss, S., et al.: Rev. Gastroenterology 16:501-509 (June) 1949.

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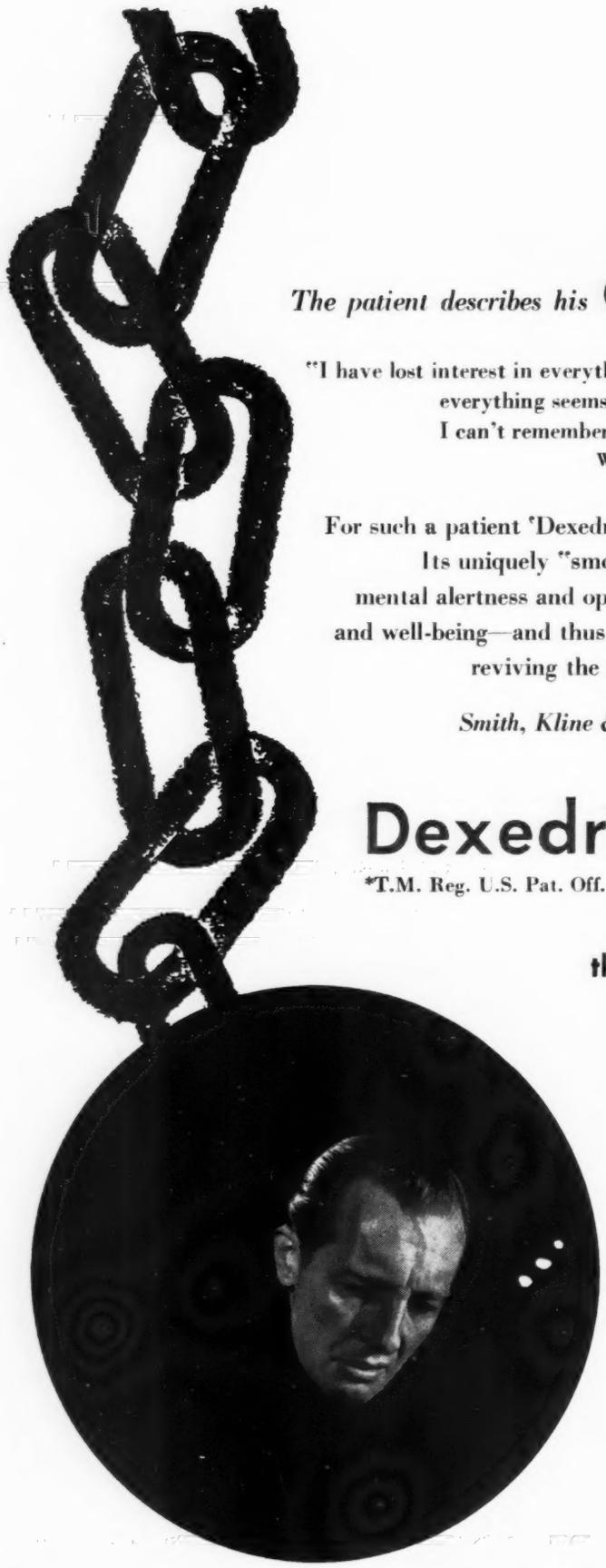

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Washburne, A.C.: Ann. Int. Med. *32*:265, 1950.

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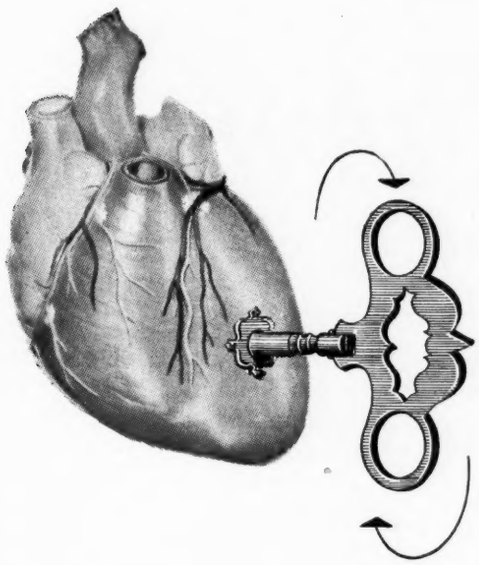

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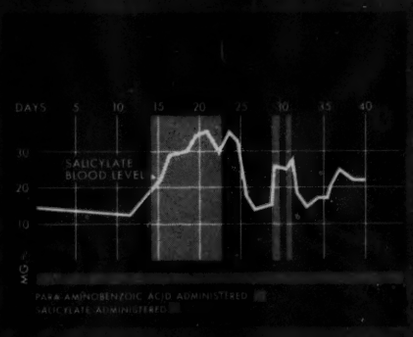

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1. Rosenblum, H. and Fraser, L. E.: Proc. Soc. Exper. Biol. and Med., 65: 178, 1947.
2. Dry, T. L., et al.: Proc. Staff Meetings Mayo Clin., 21: 497, 1946.
3. Belisle, M.: Union Med. Can., 77: 392, 1948.

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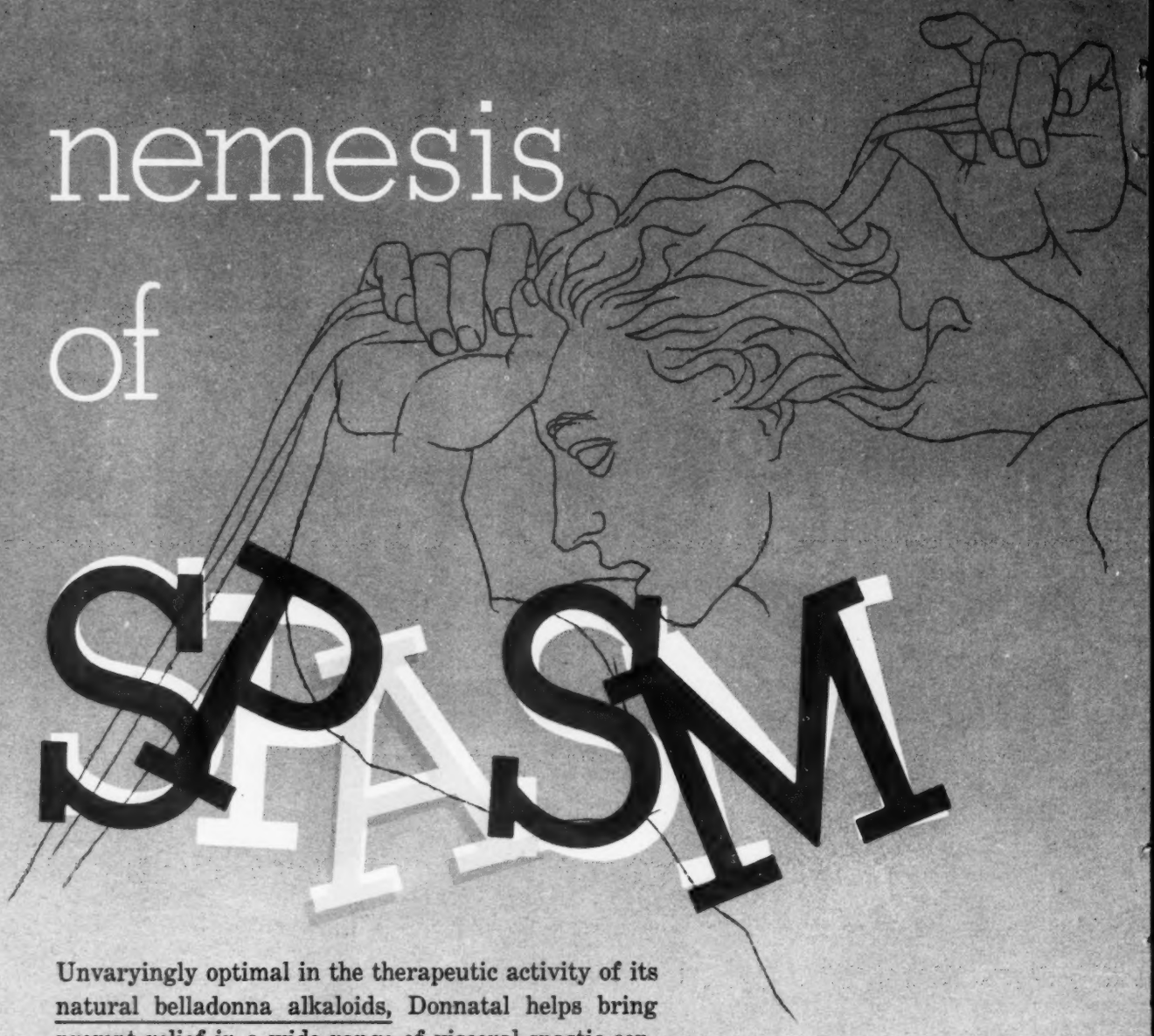

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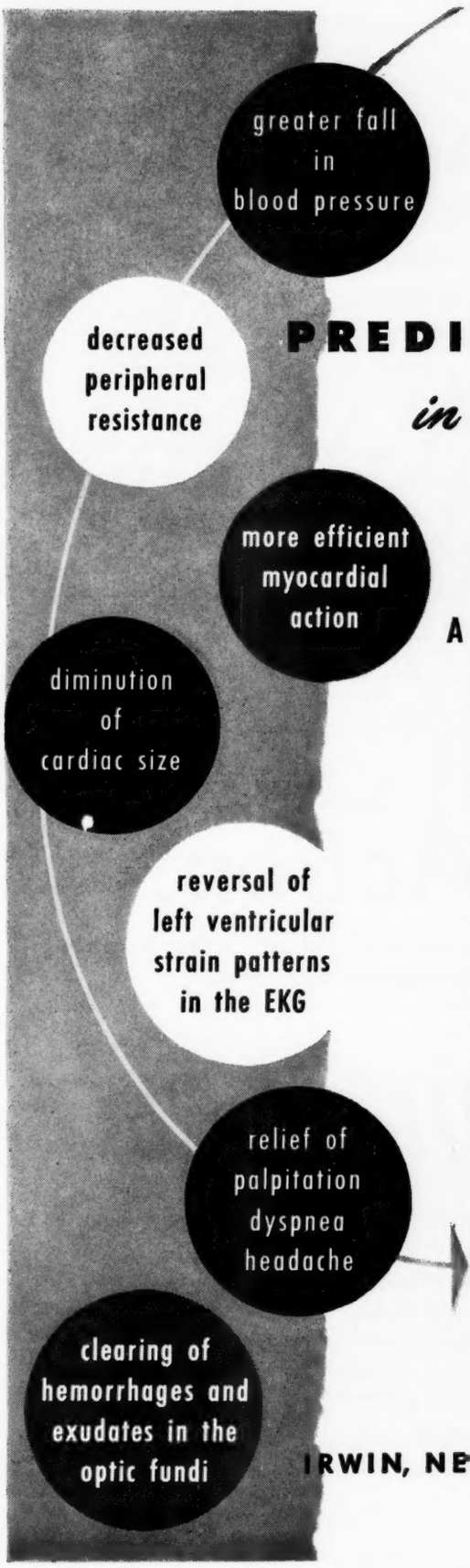

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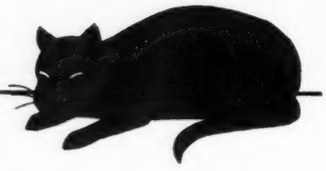

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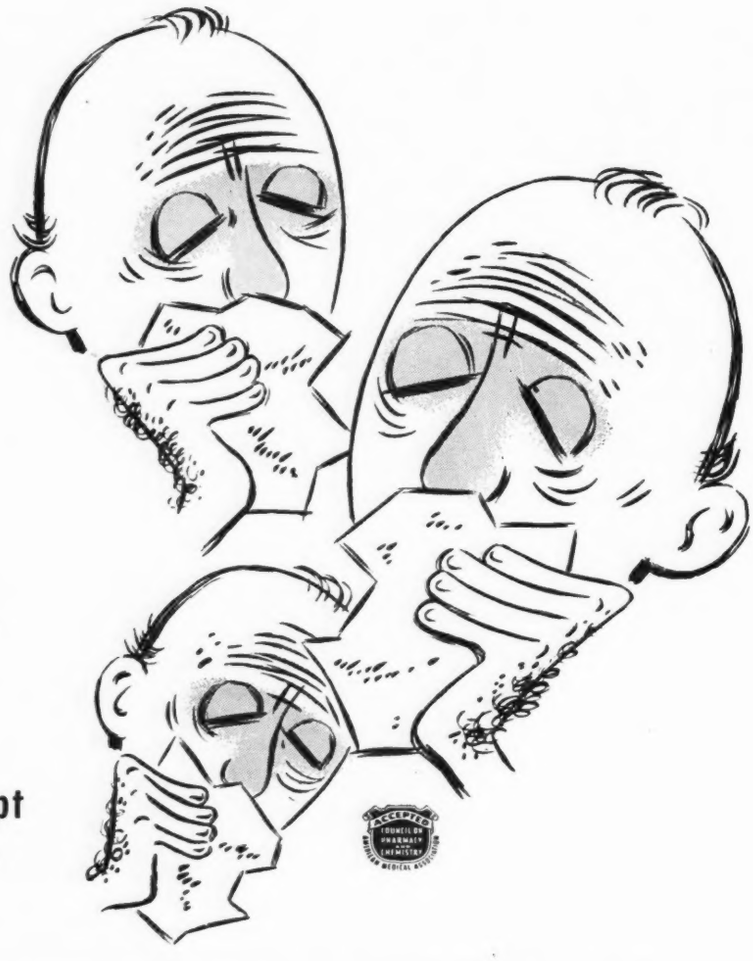

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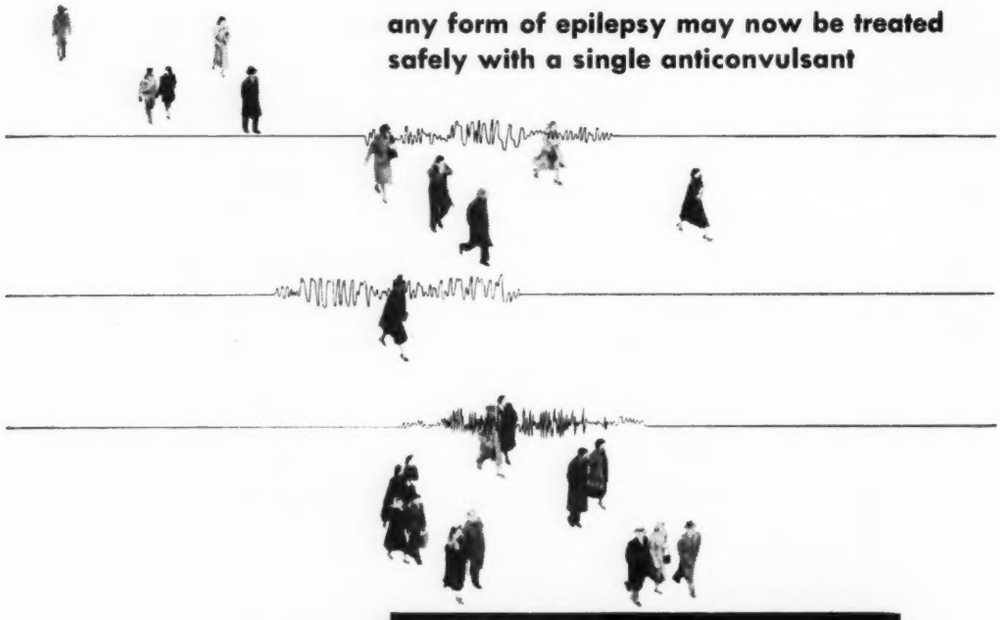

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The American Journal of Medicine

VOL. IX SEPTEMBER, 1950 NO. 3

Editorial

The Function of the Plasma Cell

PLASMA cells have attracted much attention since their morphology was precisely defined by Marschalkó in 1895. They are not found in the embryo but after birth appear in some presumably normal interstitial, lymphoid and glandular tissues. Not prominent in the histologic picture of most acute inflammations, they accumulate as a part of the cellular reaction to a wide variety of subacute and chronic infections and as a consequence of some supposedly hypersensitive states. They may be seen in and about benign tumors and in the connective tissue stroma of cancer. In the condition usually called multiple myeloma, plasmacytes or cells closely resembling them may form single or numerous tumors in the bone marrow or as myelomatosis with or without tumors may be widely scattered in the bone marrow and other tissues of the body. Occasionally they may accumulate in the circulating blood in numbers sufficient to suggest a plasma cell leukemia.

For many years interest in the plasma cells was focussed almost entirely upon their morphology and distribution with only the most casual attention to their functional significance. The association of Bence Jones proteinuria with multiple myeloma was, however, the occasion of increasing comment and led eventually to the suggestion by Magnus-Levy and others that plasma cells might actually be responsible for the production of abnormal proteins. In the meantime, clinical estimation of serum proteins revealed that many abnormal globulins quite distinct from those of the Bence Jones group are present in cases of multiple myeloma and also that hyperglobulinemia is frequent in kala-azar, lymphogranuloma venereum and other conditions in which plasma cell proliferation is obtrusive. A possible relationship between the two phemonena became apparent to several observers almost simultaneously but was stated most clearly by Bing and Plum[1] who suggested "that a comparison of the various affections in which hyperglobulinemia is found show as a common feature an augmentation of plasma cells and other cells belonging to the reticuloendothelial system." Since 1937 when this statement was made numerous observations have accumulated to substantiate the coincidence and to lend support to the thesis that some or all of the abnormal proteins of myeloma and other clinical conditions characterized by hyperglobulinemia are produced by plasmacytes or their immediate precursors. They have also suggested the idea that plasma cells might be implicated in the formation of immune globulins or antibodies.

Clinical evidence concerning the participation of plasma cells in immunity must be regarded as only circumstantial. It rests chiefly on their frequent presence in subacute and chronic infections, their appearance in the circulating blood of patients with rubella, rubeola and other exanthemas and their demonstration in isolated instances of serum sickness, sulfadiazine poi-

[1] BING, J. and PLUM, P. Serum proteins in leukopenia. *Acta med. Scandinav.*, 92: 415, 1937.

soning and other conditions ascribed to hypersensitization.

Evidence from animal experimentation, however, appears to be more convincing. For the most part, it has consisted of combined cytologic and immunologic studies following induction of the anamnestic response in rabbits and other animals by repeated injections of a wide variety of antigens which have included horse serum, typhoid vaccine, pneumococcus vaccine, streptococcus viridans, organisms of the salmonella group and Bacillus abortus. Among the outstanding contributions have been those of Bjørneboe, Gormsen and Lundquist,[2] Kolouch, Good and Campbell[3] and Fagraeus[4] whose illuminating monograph offers a summary review of the problem. Although varying somewhat in scope and method, all of these experiments have exhibited with continuing injection of antigen both a rising titer of antibody and a widespread and often very large accumulation of plasmacytes. Careful cytologic studies at successive stages in the development of immunity have revealed cells in various phases, from those closely simulating reticulum cells and young lymphocytes to the adult or Marschalko type of plasma cell. Although at present there is no agreement as to the stage at which antibody production is maximal, there can be little doubt as to the coincidence of plasmacyte response and the development of immune proteins.

Consideration of this evidence naturally raises the question of the relationship of gross hyperglobulinemia encountered in subacute and chronic infections, and states of hypersensitization to immunity and specific antibody response such as has been demonstrated in the animal experiments. Could it be that abnormal globulins of such conditions as kala-azar, lupus erythematosus, Boeck's sarcoid and lymphogranuloma venereum are antibodies produced by plasma cells in response to the individual antigens of each particular disease? The question may be raised even in relation to multiple myeloma. Is it possible that many of the cases of diffuse myelomatosis, classified in the literature as myeloma and regarded as examples of malignant new growth, represent an immunologic response engendered by as yet unknown stimuli and characterized by an excessive plasma cell response and the production of sometimes huge amounts of immune globulins? Possible examples of such myelomatoses are Carter's case[5] of persistently active trichiniasis and Robertson's cases[6] of sulfadiazine poisoning. In each instance the diffuse plasma cell infiltration was attributed initially to myelomatosis and only after further study to hypersensitization.

The implication of the rapidly accumulating clinical and experimental evidence is that the plasma cell possesses an important function in globulin formation the extent of which, however, is only outlined by existent data. At present it appears that plasmacytes are not responsible for the production of normal serum globulins inasmuch as they fail to proliferate or accumulate during intensive and prolonged plasmaphoresis.[4] If, however, they are concerned both with the formation of antibodies and the proteins of clinical hyperproteinemia, the range of their activity is extremely varied. Relatively little is known of the exact nature of the abnormal globulins in chronic infections. In multiple myeloma alone, however, they may include the Bence Jones family of proteins, the cold-precipitable proteins or cryoglobulins and a large number of substances possessing no outstanding peculiarities of temperature solubility but

[2] Bjørneboe, M., Gormsen, H. and Lundquist, F. Further experimental studies on the role of plasma cells as antibody producers. *J. Immunol.*, 55: 121, 1947.

[3] Kolouch, F., Good, R. A. and Campbell, B. The reticuloendothelial origin of bone marrow plasma cells in hypersensitive states. *J. Lab. & Clin. Med.*, 32: 749, 1947.

[4] Fagraeus, A. Antibody formation in relation to the development of plasma cells. *Acta med. Scandinav., Suppl.*, 204: 1, 1948.

[5] Carter, J. R. Plasma cell hyperplasia and hyperglobulinemia in trichinosis. The duration of larviposition. *Am. J. Path.*, 25: 309, 1949.

[6] Robertson, T. Plasmacytosis and hyperglobulinemia associated with hypersensitivity reaction. A report of two cases studied postmortem. *Am. J. Med.*, 9: 315, 1950.

identifiable electrophoretically usually as gamma or beta globulins.

In attempting to evaluate the function of plasma cells it must be emphasized that all of the rather alluring evidence concerning their participation in protein formation is as yet inferential and that no actual proof is at present available. Staining reactions for ribose nucleic acid[7] and the use of ultraviolet photography[8] have indicated the ability of plasma cells to produce protein but have not established for the cells either an exclusive or a specific function. Observations such as those of Barr, Reader and Wheeler[9] disclosing large deposits presumably of cryoglobulin within and about myeloma cells are only suggestive of production of the protein *in situ*. Furthermore, it should be stressed that although recent observations tend to validate the function of plasma cells in the production of antibodies, they have neither actually disproven the earlier claims concerning the rôle of the lymphocyte nor have they dissipated completely the confusion in identification and classification of immature cells in the bone marrow or other tissues.

Even without definite proof of specific or exclusive function, however, the rôle suggested for the plasma cell is so significant and the evidence of its reality so cogent that it cannot be ignored in the consideration of clinical states and the phenomena of immunity. Much more precise information is needed concerning not only the identification and origin of the plasmacyte but also its proliferation and distribution in a great number of infections and hypersensitive states. Increasing knowledge of the nature of the vast number of proteins now included under the designations of hyperglobulinemia and antibody formation may be expected to shed new light on the problem.

DAVID P. BARR, M.D.

[7] DEMPSEY, E. W. and WISLOCKI, G. B. Histochemical contributions to physiology. *Physiol. Rev.*, 26: 1, 1946.

[8] OLHAGEN, B., THORELL, B and WISING, P. The endocellular nucleic acid distribution and plasma protein formation in myelomatosis. *Scandinav. J. Clin. & Lab. Investigation*, 1: 49, 1949.

[9] BARR, D. P., READER, G. G. and WHEELER, C. E. Studies of cryoglobulinemia. *Ann. Int. Med.*, *32:* 6, 1950.

Clinical Studies

Effect of Schedule of Administration on the Therapeutic Efficacy of Penicillin*

Importance of the Aggregate Time Penicillin Remains at Effectively Bactericidal Levels

HARRY EAGLE, M.D., RALPH FLEISCHMAN *and* ARLYNE D. MUSSELMAN
Bethesda, Maryland

It was shown in a previous report from this laboratory[1] that when syphilitic rabbits were treated with penicillin in aqueous solution the total curative dose was strikingly reduced if the drug was given in multiple injections instead of being given in a single massive dose or in a few large doses. The interval between injections was also of major importance in determining the therapeutic outcome. Optimum results were obtained when sodium penicillin was given twice daily, and either a shorter or longer interval necessitated the administration of larger doses. In the light of these data and qualitatively similar preliminary results subsequently obtained in pneumococcal and streptococcal infections it was suggested[2] that the activity of penicillin was probably determined by the time for which the drug remained at effective concentrations, supplemented by the period for which organisms may continue to die after the penicillin itself has fallen to levels not bactericidal *in vitro*. Jawetz[21] also found multiple injections to be more effective than a single injection in the treatment of a streptococcal infection in mice, and also stressed the importance of the interval between injections on the therapeutic outcome; more recently, Schmidt, Walley and Larson[23] again found that multiple injections of sodium penicillin were therapeutically more effective than the same total dose given in a single injection. The latter workers suggested that relatively high plasma concentrations may have a beneficial effect either by prolonging the recovery period[4–6] or by effecting a greater localization of the drug in the bacteria.

It was, however, subsequently reported by Zubrod[3] and by Gibson[3a] that a single injection of aqueous penicillin was just as effective in the treatment of experimental mouse infections as that same amount of penicillin divided into multiple doses. The implication was that the total duration of effective penicillin levels was not of critical importance, in that a high concentration acting for a short time seemed just as effective as a low concentration acting over a longer period.

Additional data will be presented herein which are believed to reconcile some of the apparent discrepancies and to reinforce the thesis that the major determinant factor in the therapeutic activity in penicillin is the aggregate time, not necessarily continuous, for which the drug remains at bactericidal levels. Of importance also is the fact that after the concentration of penicillin has fallen below those effective levels the surviving bacteria do not immediately recover from the toxic effects of the drug.[2,4,5] During this prolonged recovery period the damaged but still viable organisms of some species continue to die under the impact of the body's defense mechanisms.[6,16] As will be

* From the Section on Experimental Therapeutics, Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Md.

shown, this serves to decrease the time for which penicillin should be produced at effective levels.

METHODS AND MATERIALS

Mice were inoculated with Diplococcus pneumoniae types I and III, and with group A and group B β-hemolytic streptococci. The route of inoculation, number of organisms injected and the interval between inoculation and treatment are indicated in the tables. Rabbits were inoculated intramuscularly with the same strain of group β-hemolytic streptococcus. At varying intervals after inoculation the animals were treated with sodium penicillin G in aqueous solution injected intramuscularly either as a single injection or in multiple divided doses as indicated in the tables. The size of the inoculum was usually calibrated by a microscopic count of the original culture,[7] with a simultaneous determination of the number of organisms per clump. This direct count was checked by plating out the dilution actually used for inoculation.

Unless otherwise stated the cultures were used during the logarithmic phase of growth, after two to three hours' incubation at 37°C. With such cultures one to ten organisms of the strains here used usually caused a fatal infection in mice. The group B strain of β-hemolytic streptococcus caused a fatal infection of rabbits with an intramuscular inoculum of two organisms. Because of the large number of animals used in each type of infection it was not possible to carry out all the experiments with a given organism on the same day. The experimental data summarized in each of the tables and figures are therefore a composite of a number of experiments carried out at different times. However, consistent results were obtained in repeat experiments with the same type of infection treated on the same dosage regimen; and the grouping of the data is not considered to have affected the validity of the conclusions drawn.

In Figures 1, 3 and 4 the curves relating to syphilitic infection in rabbits are based on previously published data,[1] recalculated by the method of either Litchfield and Fertig or of Kärber.[8] The curative doses of the amorphous penicillin in units/kg. have been expressed as mg./kg. on the assumption of 1,667 units/mg.

It is a pleasure to acknowledge the advice and assistance of Mr. Nathan Mantel of the Office of the Statistical Coordinator, Division of Public Health Methods, U. S. Public Health Service, in calculating from the experimental data the CD_{50} values of penicillin here reported, as well as the times for which those CD_{50} dosages had provided serum concentrations in excess of the minimal effective concentration.

	Rabbits		Mice	
Dosage mg./kg.	Slope Constants	Serum Concentration at Zero-time*	Slope Constants	Serum Concentrations at Zero-time
200			.86	329
60	.50	63	1.21	84
10	.44	9.5	1.60	20
3	.57	3.3	1.93	4.8
1	.43	.98	1.82	1.8
0.6	.52	.56	2.11	1.3
0.15	.35	.17	1.65	.15

* This is an extrapolation for purposes of analysis with no significance in terms of actual serum concentrations. Obviously, none of the penicillin had been absorbed from the site of injection at time 0.

In Figure 2 the lines drawn in the figure were fitted by the least squares method to previously published data[12] relating the log of the median serum concentration to the time elapsed since the injection. In the calculation, each median value was weighted according to its standard error. The present figure includes additional data at dosages of 0.15 and 0.05 mg./kg., and one point (one-hour level after dose of 1 mg./kg.) has been modified in the light of additional data. All serum concentrations less than 0.08 micrograms/cc. have been corrected for the inhibitory effect in the assay. The corrections for mouse serum proved to be significantly lower than those for human[13] or rabbit serum, the multiplying factors averaging 1.9 for whole serum, 1.45 for 1:2 serum and 1.19 for 1:4 serum.

The slope constants of these fitted lines (change in log concentration per hour) and the zero-time intercept of these lines (micrograms per cc.) are listed above for both rabbits and mice. Only the mouse data are shown in the figure. The 0.05 line was fitted visually.

These fitted lines permit an estimation of the average time for which the serum concentration of penicillin remains in excess of, e.g., 0.1 micrograms/cc. after its injection at varying dosage. In Figure 5 these time estimates have been plotted against the amount of penicillin

injected, both scales being logarithmic. At dosages of penicillin high in relation to the serum concentration required, the latter relationship proved to be approximately linear over a wide range of penicillin dosages. Least square lines were therefore fitted, using weights which depended on the standard errors of the estimated "penicillin-times." Separate and steeper lines were fitted for the lower dosages. In the mice data, there were small but significant departures from linearity, and in Table VI the standard errors of the estimates made from these lines have been increased accordingly.

Unless otherwise stated, "in calculating the probit relationships indicated in the tables, provisional estimates were made by Kärber's method[24] or, when indicated, by an adaptation of Kärber's method after Cornfield and Mantel.[25] (Graphic methods were used when this was not feasible.) These estimates were put through one or two cycles of computation of the maximum likelihood procedure, using the Cornfield-Mantel tables of weighted deviations and weighting coefficients. The standard errors indicated for the CD_{50} dosages have been increased to allow for significant values of chi-square where indicated. Where there were natural survivors in the absence of treatment (as with small num-

TABLE I

EFFECT OF THE SCHEDULE OF ADMINISTRATION ON THE THERAPEUTIC EFFICACY OF PENICILLIN IN MICE INOCULATED WITH PNEUMOCOCCI*

Penicillin Species	Mg./kg.	Proportion of Mice Dying When Treated with							
		Single Injection	1 Hr.	Four Injections at Intervals of				Ten Injections at Intervals of	
				3 Hr.	6 Hr.	9 Hr.	24 Hr.	1 Hr.	3 Hr.
G	512	4/20							
	256	11/20							
	128	15/20							
	64	19/20					0/20		
	32	17/20	4/20			1/20	5/20		
	16	19/20	6/19			0/20	10/20		
	8	20/20	9/20		1/20	3/20	14/20		
	4		6/20	0/20	10/20	10/20	19/20		
	2		16/20	3/20	8/20	11/20	18/20		
	1		12/20	5/20	18/20	13/20	19/20	1/15	3/20
	½		16/20	19/20	19/20	15/20		2/15	10/20
	¼		22/30	18/20		20/20		13/15	17/20
	⅛			20/20				15/15	18/20
	1/16								12/12
Slope Constants† ± Standard Error		1.61 ± 0.3	0.73 ± 0.16	3.5 ± 0.5	2.35 ± 0.4	1.61 ± 0.23	1.62 ± 0.23	4.24 ± 0.69	2.61 ± 0.49
Dose Which Cured Half of Animals (CD_{50})‡ mg./kg. ± Std. Error		256 ± 59	14.2 ± 4.9	3.4 ± 0.50	10.3 ± 1.8	8.4 ± 1.7	64 ± 13	3.8 ± 0.53	3.6 ± 0.58
X	512	3/10							
	256	9/20							
	128	11/20							
	64	17/20							
	32	19/20	1/15						
	16	18/20							
	8	19/20	3/15						
	4	20/20	7/15			0/15			
	2		5/15			3/15			
	1		9/15	0/15		5/15		1/15	1/20
	½		10/15	0/15		10/15		1/15	4/20
	¼		8/15	12/15		12/15		2/15	11/20
	⅛		13/15	14/15		15/15		5/15	19/20
	1/16			13/15				15/15	11/12
Slope Constants† ± Std. Error		1.48 ± 0.26	1 ± 0.24	4.16 ± 1.21		1.32 ± 0.27		2.15 ± 0.56	2.52 ± 0.4
Dose Which Cured Half of Animals (CD_{50})‡ mg./kg. ± Std. Error		197 ± 49	4.4 ± 1.7	1.0 ± 0.22		3.3 ± 0.96		1.1 ± 0.25	1.9 ± 0.32

* Mice were inoculated intraperitoneally with Diplococcus pneumoniae type I (0.2 cc. of a 1:200–1:500 dilution of an 18–24 hour culture at 37°C., representing 10^4–10^5 MLD). Intramuscular treatment with penicillin at the indicated dosage was begun two hours later.

† Increase in probits of survivors for each tenfold increase in dosage.

‡ Calculated by method of Litchfield and Fertig.[10]

bers of organisms injected intramuscularly in mice), the empirical responses were first adjusted by Abbott's formula for the assumed rate of natural survivors (5 to 10 per cent). In such cases the Finney procedure and tables[24] were used in the calculations."[8]

penicillin in type I and type III pneumococcal infections in mice, group A streptococcal infections of mice and group B streptococcal infections of rabbits is shown in detail in Tables I to IV, respectively. The

TABLE II

RELATIVE EFFICACY OF PENICILLIN WHEN ADMINISTERED IN SINGLE AND MULTIPLE INJECTIONS—RESULTS IN MICE INOCULATED INTRAMUSCULARLY WITH DIPLOCOCCUS PNEUMONIAE, TYPE III, AND TREATED IMMEDIATELY WITH AQUEOUS PENICILLIN G, INTRAMUSCULARLY

Total Dosage of Penicillin G, mg./kg.	Inoculum = 100 Organisms*		Inoculum = 10,000 Organisms*		Inoculum = 1,000,000 Organisms*	
	Proportion of Mice Which Died When Treated:					
	By a Single Injection	4 Times at 3-hr. Intervals	By a Single Injection	4 Times at 3-hr. Intervals	By a Single Injection	4 Times at 3-hr. Intervals
1024†					0/10	
512			1/15		2/30	
256			2/30		6/30	
128			5/45		20/30	
64			14/30		21/30	0/10
32	0/15		13/30	0/15	8/10	0/20
16	1/15	1/30	22/40	3/30		5/20
8	1/30	3/30	22/30	17/30	22/25	25/35
4	7/20	10/30	18/20	13/15		24/30
2	12/30	21/30		11/15		9/10
1	20/30	23/30		13/15		
½	26/30	27/30		15/15		
¼	27/30	13/15				
⅛	13/15					
Slope Constants‡ Std. Error	2.35 ± 0.35	2.68 ± 0.42	1.55 ± 0.19	4.37 ± 0.81	1.59 ± 0.38‖	2.79 ± 0.46
Dose Which Cured Half of Animals (CD_s^4), mg./kg.,§ ± Std. Error	1.95 ± 0.2	3.14 ± 0.52	36.5 ± 5.5	8.9 ± 0.9	106 ± 29‖	9.4 ± 1.1

* The plate counts on the 0.2 cc. inocula (containing by microscopic count 100, 10,000 and 1,000,000 organisms) ranged from 70–148, 5300–14,600, and 730,000–020,000, averaging 110, 10,100 and 900,000, respectively.

† At larger doses a significant proportion of the mice died because of the primary toxicity of the penicillin.

‡ Increase in probits of survivors for each tenfold increase in dosage.

§ The mortality in control animals receiving 10^1, 10^2, 10^4, and 10^6 organisms was 96/120, 102/127, 64/75 and 28/28, respectively. In the groups receiving 10^2 and 10^4 organisms, the empirical response to treatment was adjusted by Abbott's formula on the arbitrary assumption of 10 per cent natural survivors. In the controls 15 to 20 per cent of the mice actually survived; but significantly fewer animals, varying between 5 and 15 per cent, survived in those receiving small doses of penicillin.

‖ A significant value of chi-square has been allowed for by multiplying the standard error by $\sqrt{\frac{X^2}{\text{degrees of freedom}}}$.

EXPERIMENTAL RESULTS

Single versus Multiple Injections of Penicillin. The striking effect of the schedule of administration on the therapeutic efficacy of

results of all these experiments are summarized in Table V and most of these are shown graphically in Figure 1. Previously reported data in rabbit syphilis are also included in that figure.

In most of the experiments a relatively large number of bacteria were present in the infected animals at the time of treatment, either because a large number of bacteria (10^4–10^7) had been inoculated or because the long interval between inoculation and treatment had permitted the interim multiplication of the organisms. In every such experiment, with all the bacterial species tested, regardless of the route of inoculation or the time of treatment relative to inoculation, the curative dose of penicillin was many times greater when the drug was given in a single injection than when given in divided doses. This is shown in Figure 1. In some experiments the difference was as much as a hundred- to a thousandfold. Thus in mice inoculated with 10^6 group A β-hemolytic streptococci (Table III) the single curative dose (CD_{50}) was 51 mg./kg.; divided into four injections at three-hour intervals the total curative dose was 0.53

TABLE III

THE RELATIVE EFFICACY OF PENICILLIN G WHEN ADMINISTERED IN SINGLE AND MULTIPLE INJECTIONS TO MICE INOCULATED WITH STREPTOCOCCI*

Total Dosage of Penicillin G, mg./kg.	Inoculum = 100 Organisms†		Inoculum = 10,000 Organisms†		Inoculum = 1,000,000 Organisms	
	Proportion of Mice Which Died When Treated:					
	With a Single Injection	4 Times at 3-hr. Intervals	With a Single Injection	4 Times at 3-hr. Intervals	With a Single Injection	4 Times at 3-hr. Intervals
512‡			1/13			
256			2/30		0/20	
128			3/30		3/30	
64			12/45		11/30	0/10
32		1/30	12/30	0/15	29/30	0/10
16		4/30	17/30		16/20	
8	0/15	10/60	24/30	1/15	28/30	4/25
4	1/30	14/60	25/30	6/45		1/35
2	3/30	18/65		0/29		0/35
1	5/45	20/60		0/30		7/35
½	8/45	18/60		6/30		18/35
¼	17/45	18/43		23/30		13/15
⅛	25/30	18/30		29/30		15/15
1/16	27/30	14/15		15/15		
Slope Constants§ ± Std. Error	0.72 ± 0.1	2.05 ± 0.49¶	1.3 ± 0.16	1.92 ± 0.56¶	2.6 ± 0.71¶	3.75 ± 0.56
Dose Which Cured Half of Animals (CD_{50})‖ ± Std. Error	0.35 ± 0.088	0.26 ± 0.063¶	22 ± 3.85	0.43 ± 0.15¶	50.7 ± 12.2¶	0.53 ± 0.05

* Results in mice inoculated intramuscularly with Streptococcus pyogenes (C-203) and treated immediately with aqueous penicillin G, intramuscularly; composite of eight individual experiments carried out at different times.

† In eight individual experiments the plate counts on the 0.2 cc. inocula containing by microscopic count 100 and 10,000 organisms ranged from 45–362 and from 7200–25,500, and averaged 138 and 14,200, respectively.

‡ At doses larger than 1024 mg./kg. a significant proportion of the mice died because of the toxicity of the penicillin.

§ Increase in probits of survivors for each tenfold increase in dosage.

‖ The mortality in the control animals receiving 10^1, 10^2, 10^4 and 10^6 organisms was 63/75, 79/93, 47/50 and 42/42, respectively. The empirical response to treatment was adjusted by Abbott's formula on the arbitrary assumption of 10 per cent natural survivors in those receiving 10^2 organisms and 5 per cent in those receiving 10^4.

¶ Because of a significant value of chi-square, the standard error has been multiplied by $\sqrt{\frac{X^2}{\text{degrees of freedom}}}$.

mg./kg. In type I pneumococcal infections of mice similarly treated (Table 1) the curative doses of penicillin G on the single- and multiple-dose schedules were 256 and 3.4 mg./kg., respectively; with penicillin X the corresponding curative doses were 197 and 1 mg./kg. In syphilitic rabbits the curative dose (CD_{50}) when injections were repeated fifty times at four-hour intervals had been previously[1] shown to be a total of 360 units/kg. compared with more than 500,000 units/kg. when penicillin was given in a single injection. Further illustrating the greater efficacy of multiple injections of penicillin in these infections, dosages which cured more than 90 per cent of the animals when divided in multiple doses often failed to cure any when given in a single injection. This is shown graphically in the left hand portion of Figure 4.

An apparent exception to the generally greater activity of multiple injections was observed in those experiments in which mice were inoculated with a small number of organisms highly susceptible to penicillin and were treated immediately thereafter (portions of Tables II and III dealing with inocula of 100 organisms, and the corresponding curves in Figure 1). Under these special circumstances a single dose of penicillin was just as effective as the same total amount of drug administered in divided doses, and in some experiments was even more effective. The explanation is believed to lie in the serum concentrations afforded by various doses of penicillin. (Fig. 2.) Infections with large numbers of organisms have been shown to require a large single dose of penicillin in order to effect cure.[9] A single large dose of 60 mg./kg. of aqueous penicillin G in mice provides serum concentrations in excess of e.g. 0.1 micrograms/

TABLE IV

EFFECT OF THE SCHEDULE OF ADMINISTRATION ON THE THERAPEUTIC EFFICACY OF PENICILLIN IN RABBITS INOCULATED WITH STREPTOCOCCI*

Total Dosage of Penicillin, mg./kg.	A Single Injection	Proportion of Rabbits Dying† after Receiving Penicillin in Four Injections Repeated at Intervals of			
		1 Hr.	3 Hr.	9 Hr.	24 Hr.
512					0/4
256	0/2				1/6
128	0/6				2/6
64	2/6	1/6			6/8
32	5/6	1/6		0/2	4/8
16	4/6	4/6	0/6	0/2	5/6
8	6/6	5/6	1/6	0/6	4/4
4	5/5	6/6	3/6	1/6	3/3
2			2/6	2/6	
1			4/6	4/6	
½			6/6	6/6	
Slope Constants‡ ± Std. Error	2.83 ± 0.81	3.39 ± 1.05	2.04 ± 0.61	3.27 ± 0.97	1.7 ± 0.5
Dosage Which Cured Half of Animals (CD_{50}), mg./kg. ± Std. Error	39.5 ± 9.1	23.4 ± 5	2.24 ± 0.64	1.61 ± 0.34	68.4 ± 20.8

* Results in rabbits inoculated intramuscularly with 2000 β-hemolytic streptococci (group B) and treated after six hours by the intramuscular injection of penicillin as indicated in the table. In twelve individual experiments the plate counts on the 0.5 cc. inocula containing 2000 organisms by microscopic count varied from 940–3240, averaging 1650.

† Every one of thirty control rabbits receiving 20–2000 organisms died, as did nineteen of twenty-four receiving an inoculum of 2 organisms.

‡ Increase in probits of survivors for each tenfold increase in dosage.

cc. for approximately 2.4 hours. If this is now divided into six injections of 10 mg./kg., each injection provides that level for an aggregate period of $6 \times 1.45 = 8.6$ hours; while twenty injections of 3 mg./kg. each provide that level for $20 \times 1.38 = 28$ hours. 0.6 mg./kg. in mice provides a serum concentration in excess of 0.1 micrograms per cc. for an average period of 0.53 hours. If this is sub-divided into four doses of 0.15 mg./kg. each, the serum concentration remains in excess of 0.1 micrograms per cc.

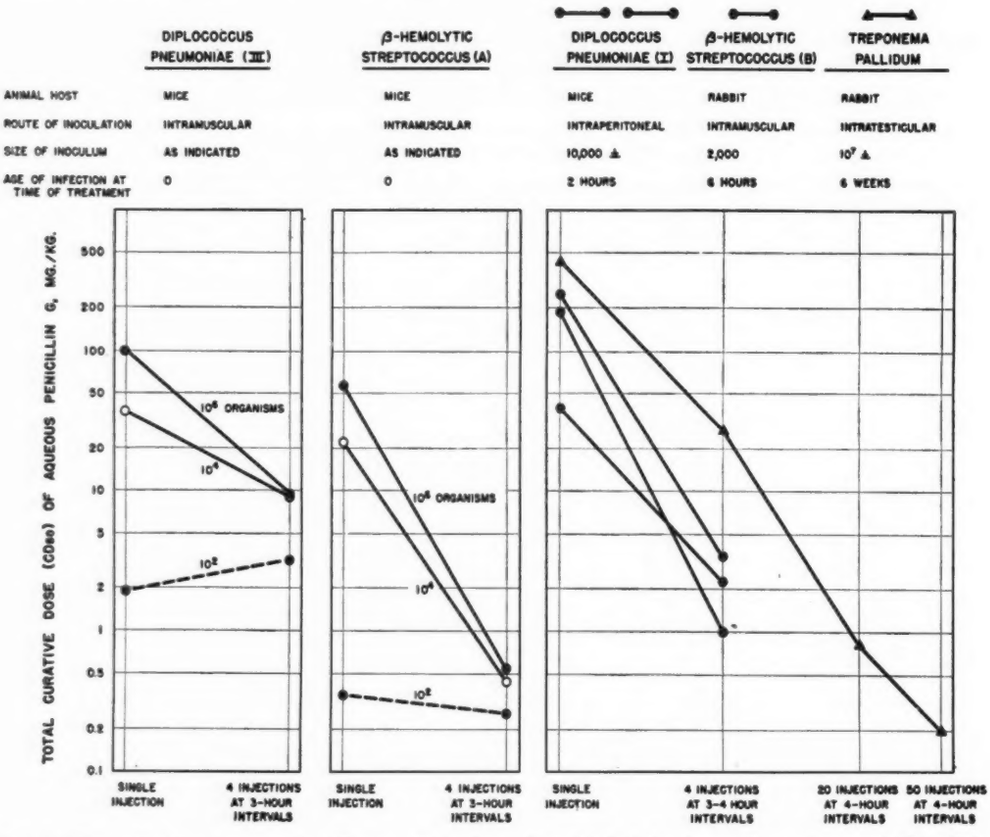

FIG. 1. The relative therapeutic efficacy of single and multiple injections of aqueous penicillin G in a number of experimental infections; (50.7 in middle block (●) incorrectly plotted).

In this instance subdivision of the penicillin into multiple injections provides effective concentrations for a longer aggregate period and the therapeutic activity is correspondingly greater. In contrast, with small inocula the provision of effective levels for an extremely brief period suffices to kill all the organisms, and this has been shown to be accomplished by a relatively small single dose of penicillin.[9] If this minimal dose is now sub-divided, one arrives at dosages so small that they provide effective concentrations of penicillin for only a relatively short period, and with further subdivision the individual dose may fail entirely to provide a therapeutic concentration. Thus a dose of for only 0.11 hours between injections to a total of 0.44 hours (Fig. 2); and when such small injections are repeated at intervals of six or nine hours, since penicillin remains at effective concentrations for only 0.11 hours, there is ample opportunity for the organisms to recover and remultiply, vitiating in large part the therapeutic effect of the preceding injection of penicillin (results with 0.15 and 0.6 mg./kg. in Fig. 8.)

The experiments of Zubrod[3] and of Gibson,[3a] who in contradiction to other investigators[2,2a,21,23] found that a single injection of sodium penicillin was as effective as the same amount divided into multiple doses, also involved a relatively small inocu-

lum and a highly susceptible organism. Their results may perhaps be explained on the basis of the foregoing considerations.

One would expect the same phenomenon even with organisms which require high concentrations of penicillin, provided that this high concentration need be maintained for only a brief period in order to effect cure. A single large dose would now be more effective than multiple small doses which fail to provide the concentration necessary for the bactericidal effect. Precisely this relationship has recently been reported by Miller, Wilmer and Verwey,[26] working with S. typhosa infections in mice.

Importance of the Time Interval between Injections of Penicillin. As seen in Table v and in Figure 3 when with a fixed total number of penicillin injections the interval between them was progressively increased, the dosage necessary for cure at first decreased to reach a minimum but thereafter again increased. In type I pneumococcal infections of white mice (Table i) when four injections of penicillin G were given at one-, three-, six-, nine- and twenty-four-hour intervals, the total curative doses were 14, 3.4, 10, 8.4, and 64 mg./kg., respectively. In a group B streptococcal infection of rabbits (Table iv) when rabbits were treated four times at intervals of one, three, nine and twenty-four hours, the curative doses were 23, 2.2, 1.6 and 68 mg./kg., respectively. Qualitatively similar results had previously been obtained in syphilitic infection of rabbits.[1] When sixteen injections were repeated at one-, two-, four- eight- to sixteen-, twenty-four-hour and three- or four-day intervals the total curative doses* were > 38, 16, 2.6 (estimated), 1.0, 2.6, and > 10 mg./kg., respectively.[1]

The fact that too short or too long an interval between injections both operate to reduce the therapeutic efficacy of aqueous penicillin is further illustrated by the widely varying proportion of animals cured when penicillin was administered at the same total dose but on different schedules of administration. (Fig. 4.) Thus when mice infected with Diplococcus pneumoniae type I were treated with four injections of penicillin at 2 mg./kg. each, but at intervals of one, three, six, nine and twenty-four hours, the number of mice surviving in each experimental group of twenty at first increased from four to seventeen and then decreased progressively from seventeen to twelve to nine to two. (Table i.) In rabbits infected with a group B β-hemolytic streptococcus and treated with four injections of 2 mg./kg. each, as the interval between injections was prolonged from one to three to nine to twenty-four hours, the proportion of animals cured at first increased from 1/6 to 5/6 to 6/6 and then fell to 0/4. (Table iv.) It had previously been shown[1] that when syphilitic rabbits were treated sixteen times at a fixed dosage of 250 units (0.15 mg./kg.) per injection, but at intervals of one, two and eight to sixteen hours, the proportion of animals cured at first increased from 0/5 to 2/5 to 7/7; with an interval of twenty-four hours the proportion of cures was 4/5;[1] and when the interval between injections was further prolonged to four days, none of the ten animals tested at this dosage were cured.[11]

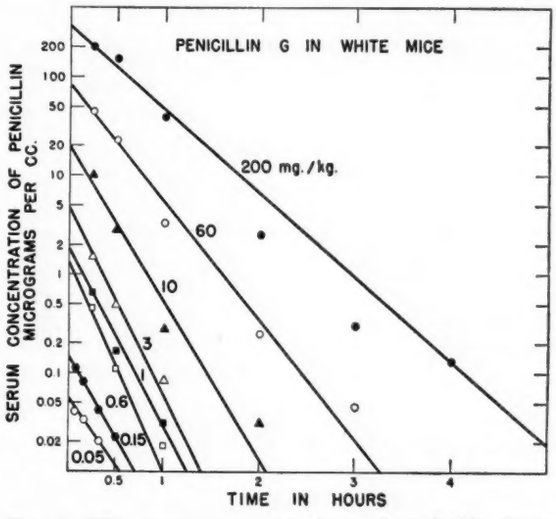

FIG. 2. The serum concentrations of penicillin G in white mice after its intramuscular injection in aqueous solution. (See page 281.)

These results are in qualitative agreement with those of Jawetz[21] working with a group

* Re-calculated from experimental data[1] by methods of Kärber and Litchfield and Fertig.[8]

TABLE V

EFFECT OF SCHEDULE OF PENICILLIN ADMINISTRATION ON ITS THERAPEUTIC EFFICACY IN PNEUMOCOCCAL AND STREPTOCOCCAL INFECTIONS—SUMMARY OF TABLES I TO IV*

Organism Inoculated	Host Species	No. of Organisms Inoculated	Route of Inoculation	Age of Infection	Curative Dose of Penicillin, mg./kg., When Penicillin was Administered in:							
					Single Injection	4 Injections at Intervals of					10 Injections at Intervals of	
						1	3	6	9	24 hr.	1	3 hr.
Type I	Mouse	10,000 ±	Intraperitoneal	2 hr.	G) 256	14	3.4	10	8.4	64	3.8	3.6
					X) 197	4.4	1.0	..	3.3	..	1.1	1.9
Diplococcus pneumoniae		100		0 hr.	2.95	....	3.1	..	...	..	...	...
		10,000	Intramuscular	0	37.5	....	8.9	..	...	..	...	...
Type III	Mouse*	1,000,000		0	106	....	9.4	..	...	..	...	...
		100*		24	140	....	7.3	..	...	..	...	...
β-hemolytic streptococcus, group A (C-203)	Mouse	100	Intramuscular	0	0.35	....	0.26	..	...	..	...	...
		10,000		0	22.0	....	0.43	..	...	..	...	...
		1,000,000		0	51	....	0.53	..	...	..	...	...
β-hemolytic streptococcus, group B	Rabbit	2,000	Intramuscular	6	40	23	2.2	..	1.6	68	...	...

* In addition, this table includes the results in an experiment in which mice were inoculated intramuscularly with 100 type III pneumococci and treated with penicillin twenty-four hours later.

All curative doses > 10 mg./kg. have here been rounded off to the nearest whole number.

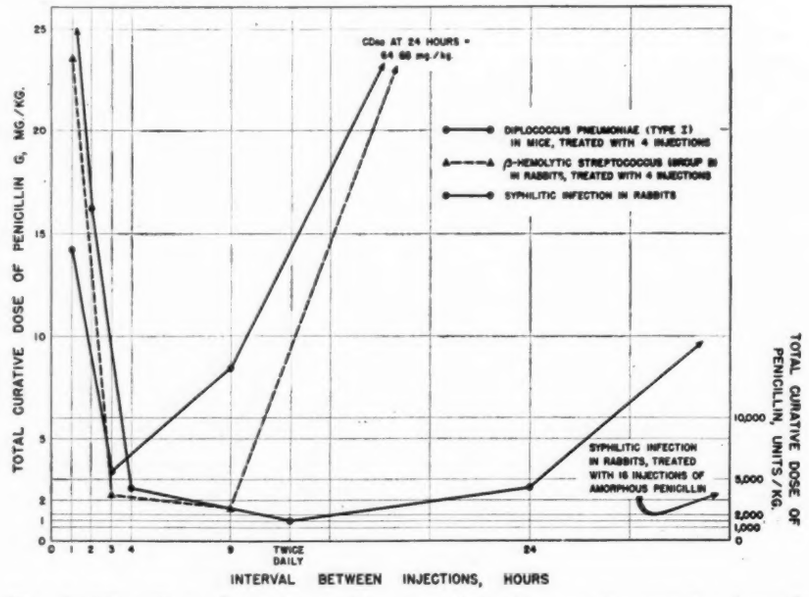

FIG. 3. The effect of the interval between injections on the curative dose of penicillin G in a number of experimental infections.

A streptococcal infection in mice, and Schmidt, Walley and Larson[23] working with a type I pneumococcal infection in rats. The fact that the therapeutic efficacy of penicillin at first increases when the interval between injections is progressively lengthened probably rests on two factors. In the first place, the total time for which penicillin remains at effective levels is thereby increased.* In addition, there is a material prolongation of the penicillin-free interval between injections during which the host defenses can, with some bacterial species, continue to dispose of organisms previously damaged by penicillin[6,16] and thus supplement the direct effect of the drug.

With too long an interval between injections, however, the bacteria which have been damaged but not yet killed by penicillin recover from the toxic effects of the drug, and resume multiplication at a normal rate.[6,16] (Fig. 8.) The longer the time interval which then elapses before the following injection of penicillin, the greater is the degree of remultiplication, partially or completely vitiating the therapeutic effect of the preceding injection.

As illustrated in Fig. 8 an appropriate increase in the dosage of penicillin counteracts the harmful effect of too long an interval between injections, and by several different mechanisms: (1) because penicillin remains at effective levels for a longer period of time, more organisms are killed; (2) because of the longer exposure to penicillin, it then takes the surviving bacteria longer to recover from its toxic effects; (3) with a longer period of effective penicillin concentrations and a longer subsequent period of recovery the residual penicillin-free interval during which the bacteria can remultiply is significantly reduced; (4) because more bacteria had been killed by the larger dose, a smaller number of viable organisms is left to multiply in the penicillin-free interval. Under such circumstances the re-multiplication of a few surviving organisms for one, two or even four generations adds but little to the absolute number of organisms which remain in the host to be killed by the following injections of penicillin.

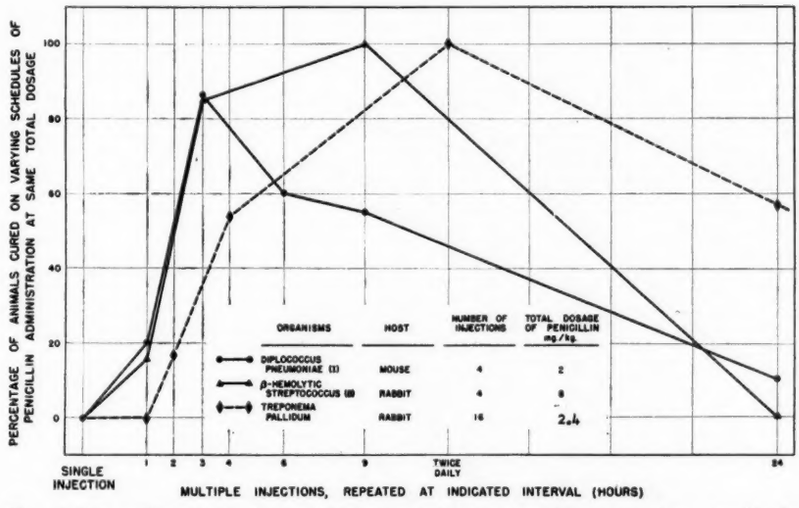

FIG. 4. The effect of the interval between injections on the proportion of animals cured by a given dosage of penicillin G.

Therapeutic Significance of the Aggregate Time Penicillin Remains at Effective Levels. The experimental results of the preceding section have been explained on the basis that the determinant factors in the therapeutic activity of penicillin are the total time for

* If a dosage of aqueous penicillin capable of supplying an effective level against a given organism for a period of three hours is repeated at hourly intervals, it is apparent that eight such injections will provide effective levels for a total of $(7 \times 1) + 3 = 10$ hours instead of $8 \times 3 = 24$. Because of the rapid rate at which sodium penicillin disappears from the blood of mice (Fig. 2), the cumulative effect of the successive injections would be so slight as to be of negligible significance.

which it remains at bactericidal levels, supplemented by the time for which, with some bacterial species, organisms damaged but not yet killed by the drug, continue to die under the impact of the host defense mechanisms even after the drug has disappeared.

dosage necessary to abort an intramuscular or intraperitoneal infection with a small number of organisms, and from that to estimate the minimum serum concentration which provides an effective level at the intramuscular or intraperitoneal focus. As

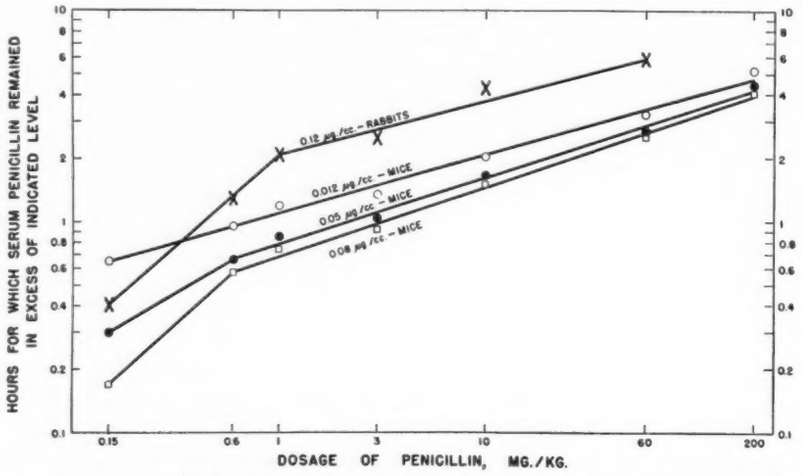

FIG. 5. Time for which the serum penicillin level remains in excess of specified effective levels after the intramuscular injections of aqueous penicillin G in various dosages. (See page 281.)

If this thesis is correct, although the curative dose of penicillin in a given infection may vary to an extraordinary degree depending upon the method of its administration, those widely disparate curative doses should have in common the fact that they provide effective levels against the particular organism for approximately the same aggregate period of time, modified only by (1) the varying opportunity for host-participation during the penicillin-free interval between injections and (2) the possible remultiplication of the organisms if that penicillin-free interval is unduly prolonged.

The direct test of that thesis is not possible since the concentration of penicillin in the tissue fluids does not lend itself to direct measurement, and a further complication is introduced by the fact that the concentrations in different organs vary widely in both magnitude and duration.[14] However, by using suspensions of procaine penicillin in peanut oil gelled with aluminum monostearate,[19] which provide well sustained serum levels of penicillin, it was possible to determine with reasonable accuracy the

shown elsewhere[15] these minimal effective serum concentrations in mice were approximately 0.012 micrograms/cc. for the C-203 strain of Streptococcus pyogenes inoculated intramuscularly, 0.05 micrograms/cc. for the type I Diplococcus pneumoniae here used, inoculated intraperitoneally, and 0.08 micrograms/cc. for the type III pneumococcus inoculated intramuscularly. In rabbits the effective serum concentration for the group B β-hemolytic streptococcus inoculated intramuscularly was estimated at 0.12 micrograms/cc.

From Figure 2 and from similar data in rabbits[12] there were obtained directly the times for which varying dosages of aqueous penicillin would provide serum concentrations in excess of these minimal effective levels.* These data are plotted in Figure 5.

* In the bactericidal action of penicillin *in vitro* there is usually a narrow range between the wholly ineffective and the maximally effective concentrations within which the rate of bactericidal action increases strikingly with the concentration of the drug.[18] *In vivo*, one would expect that within a correspondingly narrow range of concentrations, the rate of bactericidal action would vary according to the concentration of penicillin in the tissue fluid. This relationship is, however, extremely difficult

Given the curative dose per injection in a particular infection, by interpolation on the appropriate curve in Figure 5 one could now estimate the time for which that dose had provided a serum concentration effective against the particular organism. The results of those calculations are given in the last two vertical columns of Table VI.

In general, despite the wide variations in the absolute curative dose of penicillin according to the method of its administration, those curative doses provided effective serum concentrations of penicillin for essentially the same total period of time. Figures 6 and 7 show graphically the relative constancy of this time factor in a given infection, no matter how the penicillin was administered and what the absolute curative dose. The significant departures from this constant "penicillin time" are themselves illuminating.

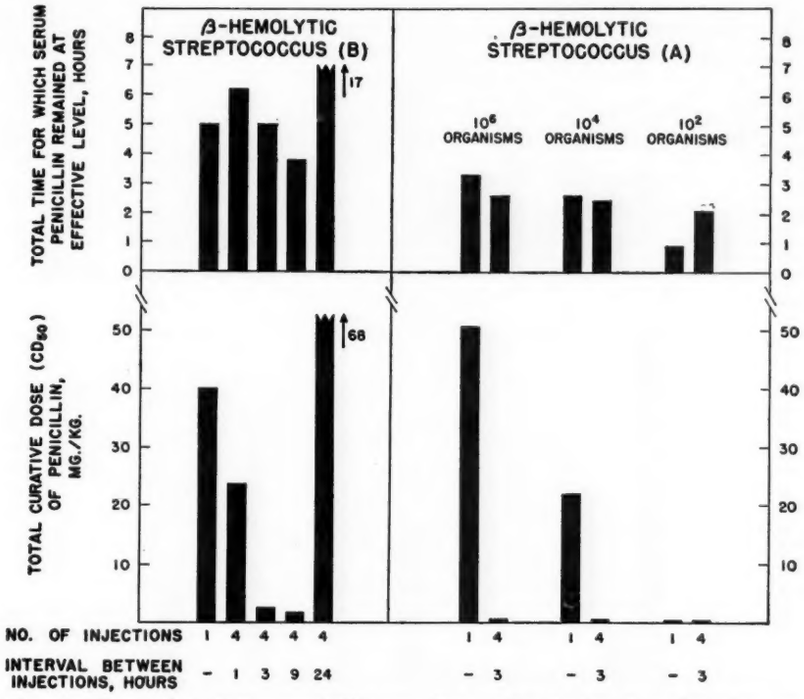

FIG. 6. The period of time penicillin remains at effective levels as the common denominator in equi-effective schedules of treatment, despite the side variations in the absolute curative dose. (Streptococcal infections in mice and rabbits, see Tables III, IV and VI.)

The first section of Table VI and of Figure 6 deals with group B streptococcal infections of rabbits. In this infection the curative doses (CD_{50}) on a single injection, and on four injections repeated at intervals of one, three, nine and twenty-four hours were 40, 23, 2.2, 1.6 and 68 mg./kg., respectively. These curative doses provided serum concentrations in excess of the minimal effective level of 0.12 micrograms per cc. for a total of 5.2, 6.3, 5.0, 3.7 and 17.4 hours, respectively. It is to be noted that as the interval between injections was progres-

to demonstrate with aqueous penicillin *in vivo*. Because of the rapid rate at which the drug then disappears from the blood and tissues, it remains in this critical intermediate range for only a few minutes. The only indication in the present experiments of a submaximal rate of bactericidal action was given by curve 0.15 in Figure 8. The penicillin times listed in Table VI are essentially the times for which the drug remained at the barely bactericidal level.[15] However, because the difference between that minimal effective concentration and that most rapidly bactericidal is usually no more than two- to three-fold,[18] and because of the rapid rate at which penicillin disappears from the serum after its intramuscular injection in aqueous solution, in most instances the times listed in the table are approximately equal to the times for which it remained at maximally effective concentrations. This factor becomes significant, however, when the CD_{50} dosage per injection is small; e.g., less than 1 mg./kg. (Fig. 2.) In Table VI no attempt has been made to correct for this complication.

sively lengthened from one to three to nine hours the time for which penicillin had to remain at effective levels in order to effect cure decreased progressively from 6.3 to 5.0 to 3.7. On the one-hour schedule of injections the penicillin serum concentration never fell below the effective level; on the three-hour schedule the serum penicillin remained at the effective level for 1.25 hours after each injection, leaving a penicillin-free interval of approximately two hours; and on the nine-hour schedule penicillin was at the effective level for only 0.94 hours, leaving a penicillin-free interval of eight hours between injections. During these penicillin-free intervals the host was continuing to dispose of organisms damaged, but not killed, by the previous action of the drug[6,16] and was thus contributing to its therapeutic effect. This continuing disposal of the damaged streptococci before they could recover and resume multiplication[16] is believed to explain the shorter time for which penicillin itself had to act in order to effect cure on this intermittent schedule of administration with this particular organism. However, when the interval between injections was further prolonged to twenty-four hours, the surviving organisms were given ample opportunity to recover and to remultiply. In consequence, larger doses of penicillin became necessary in order to effect cure, which is the same as saying a longer aggregate exposure to the drug (seventeen hours instead of the minimum of 3.7).

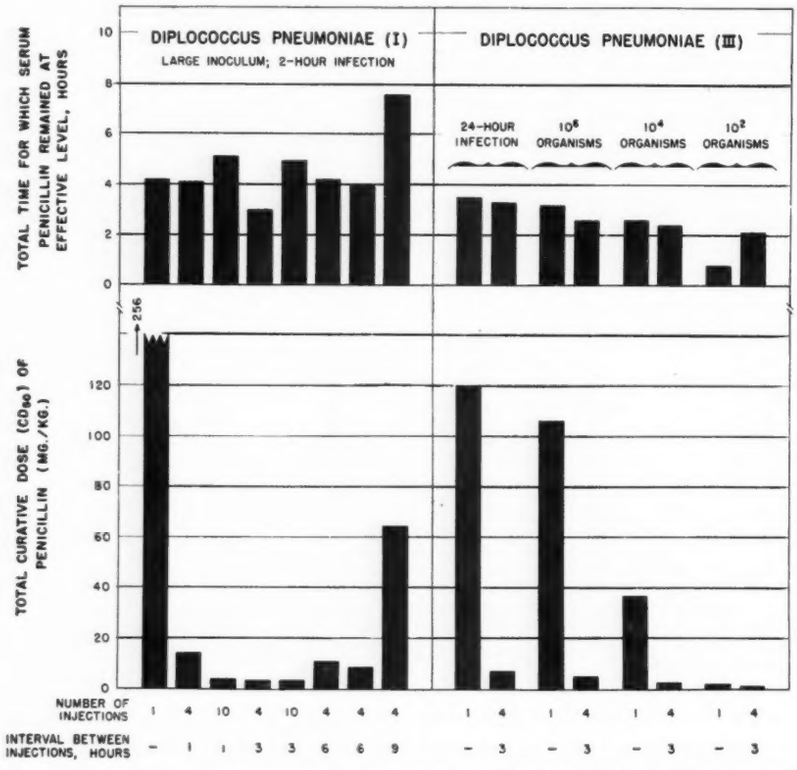

FIG. 7. The period of time penicillin remains at effective levels as the common denominator in equi-effective schedules of treatment, despite the wide variations in the absolute curative dose. (Pneumococcal infections in white mice, see Tables I, II and VI.)

The second section of Table VI and the first section of Figure 7 relates to the type I pneumococcal infection in white mice treated by eight different schedules in which the CD_{50} dosage of penicillin varied from 256 to 3.4 mg./kg. In the first seven schedules, at the CD_{50} dosage, the total time for which serum penicillin remained at the effective level of 0.05 micrograms per cc. varied only between three to five hours, and in six of the schedules between 3.9 and

5.1 hours. This minor variation in part reflects the experimental errors in the determination of the CD_{50} dose of penicillin (Table I), in the estimation of the effective serum concentration in this particular infection[15] and in the serum penicillin assays.[13] In part, however, these small differences may be real and may reflect (1) the varying degree to which the host contributes to the therapeutic action of penicillin on different schedules of treatment and (2) in the opposing sense, the varying extent to which the organisms are given an opportunity to remultiply if there is too long a penicillin-free interval between injections.

The last two sections of Table VI describe

TABLE VI

AGGREGATE TIME FOR WHICH CURATIVE DOSES OF AQUEOUS PENICILLIN G PROVIDED EFFECTIVE SERUM CONCENTRATIONS OF PENICILLIN, CONSIDERED IN RELATION TO THE METHOD OF ADMINISTRATION

Type of Infection	Effective Serum Concn. micrograms per cc.[15]	Method of Penicillin Administration		Curative Dose (CD_{50}), mg./kg.		Hours for Which CD_{50} Dose of Penicillin Provided Serum Concn. in Excess of Minimal Effective Level*	
		No. of Injections	Interval between Injections (hr.)	Total	Per Injection	After Each Injection ± Std. Error†	During Total Course of Treatment
Group B β-hemolytic streptococcus in rabbits treated with penicillin 6 hr. after intramuscular inocculation with 2000 organisms (Table IV)	0.12	1		39.5	39.5	5.24 ± 0.83	5.2
		4	1	23.4	5.85	3.25 ± 0.31	6.3
		4	3	2.24	0.56	1.24 ± 0.32	5.0
		4	9	1.61	0.40	0.93 ± 0.20	3.7
		4	24	68.4	17	4.25 ± 0.58	17
Type I, pneumococcal infection in mice treated with penicillin 2 hr. after heavy intraperitoneal inoculation (Table I)	0.05	1		256	256	4.4 ± 0.38	4.4
		4	1	14.2	3.55	1.16 ± 0.13	4.2
		10	1	3.8	0.38	0.51 ± 0.05	5.1
		4	3	3.4	0.85	0.74 ± 0.05	3.0
		10	3	3.6	0.36	0.49 ± 0.05	4.9
		4	6	10.3	2.58	1.05 ± 0.07	4.2
		4	9	8.4	2.10	0.98 ± 0.072	3.9
		4	24	64	16	1.86 ± 0.13	7.4
Type III pneumococcal infection in mice treated after intramuscular inoculation‡ with varying numbers of organisms (Table II)		Site of inoculum					
		100‡	1		140	140	3.49 ± 0.27	3.5
			4	3	7.3	1.82	0.73 ± 0.06	3.3
	0.08	10^6	1		106	106	3.18 ± 0.31	3.2
			4	3	9.4	2.35	0.90 ± 0.05	3.6
		10^4	1		36.5	36.5	2.23 ± 0.13	2.2
			4	3	8.9	2.22	0.88 ± 0.04	3.5
		10^2	1		1.95	1.95	0.85 ± 0.05	0.85
			4	3	3.14	0.78	0.63 ± 0.04	2.5
Group A β-hemolytic streptococcus infection in mice treated immediately after intramuscular inoculation with varying numbers of organisms (Table III)	0.012	10^6	1		50.7	50.7	3.21 ± 0.24	3.2
			4	3	0.53	0.132	0.64 ± 0.03	2.6
		10^4	1		22.0	22.0	2.56 ± 0.14	2.6
			4	4	0.43	0.108	0.60 ± 0.07	2.4
		10^2	1		0.35	0.35	0.83 ± 0.07	0.83
			4	3	0.26	0.065	0.52 ± 0.04	2.1

* Minimal effective serum concentrations taken as 0.012 micrograms/cc. for group A streptococcal infection, 0.05 micrograms/cc. for type I pneumococcal infection, 0.08 micrograms/cc. for type III pneumococcal infection and 0.12 micrograms/cc. for group B streptococcal infection.[15]

† This standard error takes cognizance of the standard error of the estimated CD_{50} doses of penicillin (Tables I-IV), as well as the standard error of the serum concentration data indicated in Figures 2 and 5.

‡ One group of animals in this series was treated twenty-four hours after inoculation with 100 organisms; the other three groups were treated immediately after inoculation.

the experiments with type III pneumococci and group A streptococci. (Figs. 6 and 7.) Varying numbers of organisms had been injected, and for each inoculum the curative dose had been determined when penicillin was given as either a single injection or divided into injections at three-hour intervals. (Table II and III.) For a given inoculum the curative doses on these two schedules differed as much as a hundredfold; but the aggregate times for which they provided the effective serum concentration of penicillin were again essentially the same. In mice treated immediately after inoculation with 10^6 pneumococci the penicillin times on the single- and multiple-injection schedules were 3.2 and 3.6 hours, respectively; in a twenty-four-hour infection, 3.5 and 3.3 hours; and with an inoculum of 10^4 organisms, 2.2 and 3.5 hours. In mice inoculated with streptococci the penicillin-times in the single- and multiple-dose schedules were, respectively, 3.2 and 2.6 hours with an inoculum of 10^6 organisms, and 2.6 and 2.4 hours with an inoculum of 10^4 organisms.

The only significant deviation from this constant "penicillin time" was in mice receiving small inocula (100 organisms). In such animals the single injection was apparently more efficient, an estimated exposure to effective concentrations of penicillin for only 0.85 hours and 0.83 hours sufficing to abort the pneumococcal and streptococcal infections, respectively; while when four injections were repeated at three-hour intervals, it was necessary to provide effective concentrations for 2.5 and 2.1 hours. A partial explanation may be the fact that bacteria which have been exposed only briefly to lethal concentrations of penicillin rapidly recover from its toxic effects to resume multiplication at a normal rate both *in vitro*[6] and *in vivo*.[16] Thus in mice inoculated with 100 group A streptococci a single dose of penicillin at 0.35 mg./kg., providing the effective serum concentration for 0.8 hours, sufficed to abort infection in half the animals. (Tables III and VI.) However, four three-hourly doses of 0.016 mg./kg., each providing the effective level for an estimated period of 0.2 hours, were wholly ineffective. In the penicillin-free interval of 2.8 hours the organisms would have recovered and remultiplied to an even greater extent than is indicated by the 0.15 mg./kg. curve of Figure 8. It would therefore be necessary to expose the bacteria to penicillin for a longer period, thus killing more organisms and prolonging the recovery period of the survivors. In both pneumococcal and streptococcal infections with 100 organisms this was accomplished by prolonging the "penicillin time" afforded by each injection to approximately 0.5 hours.

A further consideration may be the fact that in these small inoculum infections, aborted by small dosages and threshold concentrations of penicillin, the "penicillin time" may be shorter on a single injection because it provides *maximally* effective concentrations of penicillin; while on subdivision of the dose only the barely bactericidal level is afforded.*

The longer time required to cure syphilitic infections in rabbits as compared with experimental pneumococcal or streptococcal infections is probably related to the much slower rate at which treponemas are killed by penicillin. With the strains of pneumococci and streptococci here studied 99.9 per cent of the organisms are killed *in vitro* within two to five hours[18] and at essentially the same rate *in vivo*.[16] In contrast, with cultured treponemas it required seventeen to twenty-five hours to kill 99.9 per cent *in vitro;*[18] and pathogenic T. pallidum begins to disappear from a primary lesion in rabbits only four to six hours after the administration of even large doses of penicillin. In consequence and despite the fact that T. pallidum is exquisitely sensitive to penicillin in terms of the concentrations necessary to kill, those minimal concentrations must be maintained for a much longer period of time in order to effect cure.

Rate at Which Bacteria Are Killed by Penicillin in Vivo in Relation to the Serum Concentration of Penicillin. If the total time for which penicillin remains at effective levels

* See footnote on page 290.

is the primary determinant of its therapeutic activity, the rate at which bacteria are killed should be independent of the dosage of the drug and of its concentration in the body fluids, provided only that the latter is in excess of the concentration maximally

Each point in the figure is the median percentage of organisms surviving at the indicated time, based on groups of four to ten mice. Despite the large variation between individual mice, the trend of the data was consistent. As seen in Figure 8 the initial

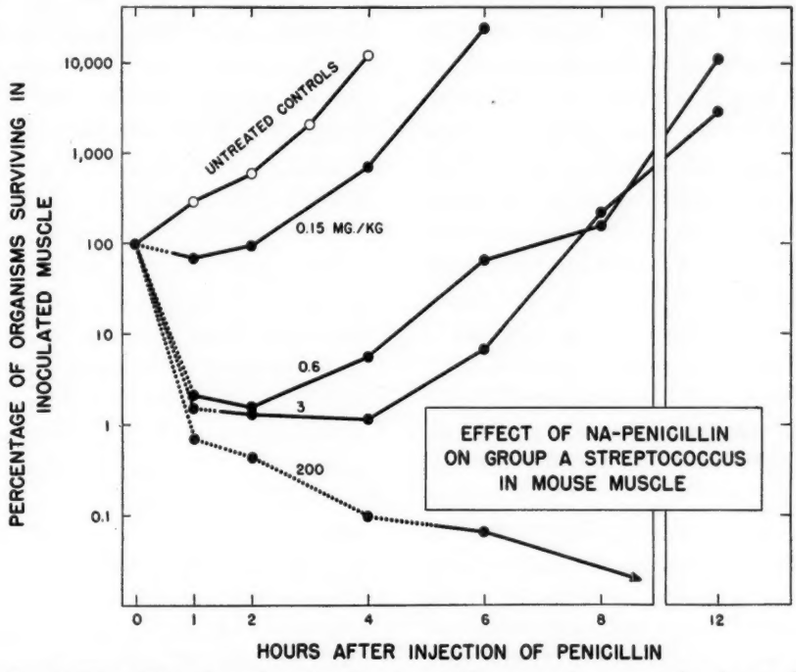

FIG. 8. The effect of varying dosages of penicillin on an intramuscular depot of streptococci.[16] Twenty thousand group A organisms were inoculated intramuscularly and penicillin was immediately injected into the opposite leg. The number of viable organisms remaining was determined by emulsifying the muscle in a Waring blendor and plating out. Each point in the figure is the median of four to ten determinations in as many individual mice.

effective against the particular organism. A large dose should be more effective than a small dose, not because it works faster* but because it continues to act for a longer period of time. This was found to be the case.

Mice were inoculated intramuscularly with 20,000 organisms and were immediately treated in the opposite leg muscle with a single injection of aqueous penicillin. At varying intervals thereafter, the inoculated muscle was removed, emulsified in a Waring blendor and the number of viable organisms in the tissue emulsion determined by plate counts. The results of a number of experiments with the C-203 strain of group A streptococci are summarized in Figure 8.

rate at which bacteria died under the impact of penicillin was independent of the dosage of penicillin and thus of its concentration in the serum and tissue fluids, provided only that that level was in excess of the concentration necessary to kill the particular organism at the maximal rate. Streptococci died no faster at a dosage of 200 mg./kg., providing a fifteen-minute level of 200 micrograms/cc., than they did at 0.6 mg./kg., providing a fifteen-minute level of 0.45 micrograms/cc.

There was thus no indication either in the experiments of Figure 8 or in similar experiments with Diplococcus pneumoniae type I, Diplococcus pneumoniae type III or a group B β-hemolytic streptococcus to

* See footnote on page 290.

be reported in detail in a following paper,[16] that a large dose of aqueous penicillin serves to "prime" the bactericidal process *in vitro*.[26] The major difference between massive doses of penicillin and doses barely bactericidal consisted in the time for which that bactericidal effect was continued. Thus, as shown in Figure 8, at a dosage of 200 mg./kg. the serum concentration remained in excess of the estimated minimal effective concentration of 0.012 micrograms/cc. for five hours. This is indicated by the dotted portion of the lowest curve; and the bacteria continued to die rapidly for that entire period. At 3 mg./kg. the serum penicillin remained in excess of that level for 1.5 hours and the rapid bactericidal action continued for approximately that same time. At 0.6 mg./kg. the effective concentration of 0.01 micrograms/cc. was provided for one hour and the bactericidal action was correspondingly brief. At 0.15 mg./kg. the serum penicillin concentration was in excess of 0.012 micrograms/cc. for less than forty-five minutes and there was correspondingly only a short-lived effect against the streptococcus.

The effect of the dosage on the duration of the post-penicillin recovery period will be discussed in a following paper.[16]

COMMENTS

Total "Penicillin Time" as the Common Denominator in Equi-effective Treatment Schedules. It has here been shown in a number of experimental infections (β-hemolytic streptococcal infections in mice and rabbits, type I and type III pneumococci in mice, and T. pallidum in rabbits) that the curative dose of penicillin may vary to an extraordinary degree, in some instances more than a hundredfold, according to the method used for its administration. However, these widely disparate but equi-effective curative doses have in common the fact that in a given infection they provide the effective concentration of penicillin for essentially the same aggregate period of time, modified only by the factors discussed in the following paragraph.

Continuous versus Discontinuous Treatment: The Importance of the Penicillin-free Interval between Injections. The time between injections for which the penicillin is at less than the effective concentration is, however, also of importance, and operates in two opposing directions to modify the therapeutic action of the drug. For a significant period of time after penicillin itself has fallen to ineffective levels the surviving but damaged organisms fail to multiply, and instead with some species remain susceptible to the host defenses.[6,16] This continuing death of the organisms is thus an aftermath of penicillin action, and in the case of group B streptococcus here studied contributes materially to the total therapeutic action of the drug.[16] With other organisms, such as the type III pneumococcus and the group A streptococcus, the host factor was only slight or not demonstrable; but even in such cases the organisms did not multiply *in vivo* for a number of hours after exposure to penicillin. Eventually, however, the organisms do recover from the toxic effects of the drug and resume multiplication. The longer retreatment is then delayed the greater is the degree to which remultiplication during the penicillin-free interval counteracts the effect of the preceding treatment

The degree to which the penicillin-free interval can be prolonged without seriously affecting the therapeutic effect of the preceding infections varies with a number of factors: (1) The danger is obviously greater with a rapidly multiplying organism than when the organisms multiply only slowly. As a case in point, in syphilitic infection in which the organism has a generation time *in vivo* of approximately thirty hours,[27,28] even an eight- to sixteen-hour penicillin-free interval between injections had no harmful effect. (2) The concentration of penicillin to which the organisms had been exposed, and the duration of that exposure, affect the time required for the bacteria to recover *in vitro*;[6] a similar relationship has been observed *in vivo*.[16] (3) With bacterial species which recover from the toxic effects of penicillin only slowly, the interval can safely be much longer than with species which recover rapidly. An instructive contrast is afforded by groups A and B strep-

tococci here studied. The group A organism recovers in mice after approximately three to four hours,[16] and with prolongation of the interval beyond this period larger doses of penicillin or a longer duration of treatment become necessary for cure. The group B organism in rabbits does not recover for approximately twelve hours;[16] accordingly, a nine-hour interval between injections had no adverse effect on the outcome of treatment, while with a twenty-four-hour interval much larger doses of penicillin were necessary for cure. (4) Finally, the smaller the number of organisms which survive the preceding treatment the less harm is done by remultiplication for one, two or four generations. If the bacterial population in the infected host had been reduced from 10^9 to 10 organisms, even after their complete recovery several hours would elapse before their remultiplication became of serious import. However, if the number of bacteria had been reduced only from 10^9 to 10^7 organisms, remultiplication for even a few generations could have a serious effect on the evolution of the infection.

The fact that bacteria remain static in number or may continue to die for a considerable period after penicillin has fallen below the bactericidal level constitutes an important safety factor in the therapeutic use of penicillin and makes possible its discontinuous administration.[2,20–23] This does not, however, mean that such discontinuous treatment, with intervals during which penicillin falls below effective levels, is to be preferred to methods of treatment which assure the maintenance of maximally effective concentrations at the focus of infection. It is a reasonable presumption that most bacteria die much faster under the combined impact of penicillin and the body's defense mechanisms than they do as a result of the latter alone. A schedule of treatment which provides the maximally effective bactericidal concentration at the focus of infection continuously should therefore be more rapidly effective than a discontinuous schedule.

"*Adequate*" *Penicillin Dosage.* The foregoing discussion points up the fact that adequate dosage with penicillin has at least two parameters. One is the concentration attained at the focus of infection, and the other is the total time for which that concentration is provided. In experimental infections with an intramuscular, subcutaneous or intraperitoneal inoculum, and treated soon after inoculation, a serum concentration approximately two to four times greater than that necessary to kill the organism at the maximal rate *in vitro* usually sufficed also to kill it at the maximal rate *in vivo;*[15] and massive doses, which provide concentrations several hundred times greater, did not further accelerate the rate of penicillin action. (Fig. 8.)[16] Further, in eight of nine bacterial strains studied the toxic effect of penicillin on the organisms (manifested by the delayed resumption of multiplication upon removal of the drug) proceeds just as rapidly at this maximally bactericidal concentration as it does at concentrations several thousand times greater.[6] By the same token, penicillin suspended in absorption-delaying vehicles which provide a reasonably sustained but low level should be just as effective as multiple injections of aqueous solution, provided only that the absolute concentration attained at the focus of infection is in excess of that necessary to kill the particular organism at the maximal rate.

The latter reservation with respect to the therapeutic efficacy of penicillin in absorption-delaying vehicles is an important one. A dosage of procaine penicillin in oil which provides serum concentrations in excess of 0.2 micrograms for the first two hours, 0.1 micrograms per cc. for the following four hours and 0.05 micrograms per cc. for the next eight hours would be highly effective against most strains of β-hemolytic streptococci. It would, however, provide effective concentrations for only four hours against an organism which requires 0.2 micrograms per cc. and would be wholly ineffective against a Streptococcus fecalis which requires a serum concentration of 4 to 8 micrograms/cc. in order to provide the effective concentration at the focus of infection.

The practice of incorporating a small amount of sodium penicillin in such pre-

parations (the water-soluble sodium salt to provide high concentrations over a short time, and the less soluble, more slowly absorbed procaine salt to provide low concentrations over a long time) would appear to add but little to their therapeutic efficacy. With organisms for which the procaine penicillin provides adequate concentrations the short-lived high concentrations afforded by the sodium salt are unnecessary and do not accelerate the death of the organisms. On the other hand, with organisms which are not affected by the low concentrations of the procaine salt, the sodium-procaine mixture would be effective only for the time that the sodium salt provides the high concentrations necessary for the particular organism and treatment would therefore have to be repeated as often as if one were using the sodium salt alone.

The foregoing discussion is based on findings in acute experimental infections. In an area of chronic inflammation with a walled-off foci of infection only slowly equilibrated with the serum and the surrounding tissue fluids it may be necessary to sustain the serum concentration at a relatively higher level for a longer period of time in order to assure the presence of the effective concentration at the actual focus of infection.

The total time for which that effective concentration must be provided at the focus of infection in order to effect cure depends on a number of factors. One is the rate at which the particular organism can be killed by the drug. It is apparent that a species of which 99.9 per cent can be killed in two hours will require a shorter period of treatment than one requiring twenty-four hours to achieve the same purpose. The number of organisms in the infected animal is a further consideration, for the greater that number the longer the time the organisms must be exposed to the drug.[9]

The problem of adequate treatment with penicillin in man is complicated by the repeated observation that the provision of adequate concentrations for what should be a sufficiently long period of time does not always ensure cure. No satisfactory explanation has yet been offered for the fact that in some cases of subacute bacterial endocarditis, with an organism which is highly sensitive to penicillin *in vitro*, the provision of serum concentrations of 10 micrograms/cc. or higher maintained for days and even weeks may fail to effect cure. In the case of early syphilitic infection also, large doses of penicillin administered over a period of two or four weeks may fail to effect cure despite the fact that the organism is killed by concentrations of less than 0.005 micrograms per cc.[15] At least in the latter instance it seems unlikely that the treatment failure is due to the presence of resistant organisms which require higher concentrations of penicillin than had actually been provided. Some of these treatment failures may be related to the fact that bacteria are susceptible to the lethal action of penicillin only in an environment conducive to growth and multiplication. It is conceivable that at a focus of infection poorly supplied with nutrient material the organisms may be multiplying so slowly that the drug is paradoxically inactive.

SUMMARY AND CONCLUSIONS

1. The curative (CD_{50}) dosage of aqueous penicillin in a number of experimental infections varies within wide limits, as much as a thousandfold, according to the schedule of its administration.

2. In a given infection these widely disparate curative doses provide effective concentrations of penicillin for essentially the same aggregate period of time, modified only by the varying effects of the penicillin-free interval between injections; and that common denominator of "penicillin time" is suggested as the primary determinant of its therapeutic activity.

3. The rate at which bacteria die under the impact of penicillin *in vivo* is independent of its absolute concentration, provided only that the latter is in excess of the level which in vitro kills the particular organism at the maximal rate. Large doses of penicillin are more effective than smaller doses primarily because of the longer time during which they provide that effective concentration.

4. A penicillin-free interval between injections modifies the therapeutic activity of the drug in two opposing senses. For a variable period of time after the penicillin itself has fallen to ineffective levels, the body may in some cases continue to dispose of bacteria damaged but not yet killed by the drug and thus contributes to its therapeutic action by reducing the time for which penicillin itself need act on the organisms. Eventually, however, the organisms recover from the toxic effects of the preceding injection and resume multiplication. An unduly long penicillin-free interval between injections therefore necessitates larger doses of penicillin and the provision of effective concentration for a longer aggregate time.

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Treatment of Rickettsialpox with Aureomycin*

HARRY M. ROSE, M.D., YALE KNEELAND, JR., M.D. *and* COUNT DILLON GIBSON, M.D.
New York, New York

EARLY studies of aureomycin indicated that this antibiotic not only had a wide range of antibacterial activity but also possessed remarkable antirickettsial properties. Wong and Cox[1] found that aureomycin had a marked therapeutic effect on infections with rickettsiae of the typhus fever, spotted fever, scrub typhus and Q fever groups in chick embryos, mice and guinea pigs. Anigstein and his associates[2] also reported that the antibiotic readily protected guinea pigs which were infected with strains of epidemic typhus and Rocky Mountain spotted fever rickettsiae. The significance of these observations with respect to the treatment of certain rickettsial infections in man has since been amply demonstrated. Aureomycin has been found to be consistently effective in the treatment of Rocky Mountain spotted fever by Bryer et al.,[3] Cooke,[4] Ross et al.[5] and Harrell et al.;[6] in tick-bite fever by Gear and Harington[7] and by Hildick-Smith;[8] in typhus by Knight et al.;[9,10] in Brill's disease by Shoenbach[11] and by Blumberg et al.;[12] and in Q fever by Lennette et al.[13] In addition Woodward[14] states that the United States Army scrub typhus team has used aureomycin in that disease with results similar to those in patients treated with chloramphenicol.

There remains one type of human rickettsial disease, rickettsialpox, in which the therapeutic effect of aureomycin has not been definitely established up to the present time. This disease is a novel infectious entity caused by Rickettsia akari, a new species which is serologically related to the spotted fever group of rickettsiae.[15] The only known endemic focus of infection is New York City and nearly all cases of rickettsialpox have been observed in that metropolitan area. However, a few cases have been seen in outlying localities among commuters or visitors who in all likelihood contracted the infection while in the city. The clinical manifestations, laboratory findings and methods for the specific diagnosis of the disease have been adequately described elsewhere[16] and will not be reviewed here. In the present report the results of aureomycin therapy in eight proved cases of rickettsialpox are set forth in some detail. Two of these cases have been briefly mentioned in a previous publication.[16] Observations are also presented concerning the possible suppressive effect of aureomycin on the immunologic response to infection by R. akari.

METHOD OF STUDY

The patients were all hospitalized in the Presbyterian Hospital, the Babies Hospital or the Harkness Pavilion of the Columbia-Presbyterian Medical Center. Acute phase blood specimens were collected aseptically by venipuncture, usually within twenty-four hours after the patients were admitted, and the serums and clots were separated by centrifugation. The clots were quickly frozen in sealed glass tubes and stored in a carbon dioxide ice box at approximately minus 70°C.; the sterile serums without added preservative were kept in an ordinary electric refrigerator at 4°C. Specimens of convalescent serum were obtained from all patients from two to six weeks after the initial bleedings and the paired serums were then tested simul-

* From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y. Aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

taneously for specific rickettsial antibody by the complement fixation method. The technic used was a modification of the Kolmer test, using serial twofold dilutions of serum, two exact units of complement and a fixation period of one hour at 37°C. The antigen was of the soluble type and was prepared according to Method No. 1 of Topping and Shepard[17] from the yolk sacs of chick embryos infected with a strain of R. akari (McCauley) previously isolated in this laboratory. The results of the complement fixation tests were expressed as the highest dilution of the patient's serum that gave at least 2 plus fixation, following addition of sensitized sheep erythrocytes and further incubation at 37°C. until serum and antigen controls showed complete hemolysis.

In those cases in which no specific immune response was detected by the complement fixation test, an attempt was made to isolate rickettsiae from the frozen blood clots. The clots were rapidly thawed at 37°C. and ground up in a sterile mortar with about 10 cc. of nutrient broth. After brief centrifugation to remove gross particles, each clot suspension was injected in 1.0 cc. amounts intraperitoneally into ten albino mice averaging 15 to 18 gm. in weight. The animals were observed carefully for signs of illness, as previously described,[16] and were sacrificed from seven to nine days after inoculation. Smears of liver, spleen and peritoneal exudate were stained by Macchiavello's method and examined microscopically for rickettsiae. Portions of liver and spleen found or suspected to contain rickettsiae were ground up in broth and these suspensions were injected intraperitoneally into fresh mice and into the yolk sacs of seven day old chick embryos. By this means, on the first or on subsequent passages, strains of R. akari were recovered from the blood of all three of the patients in this series in whom isolation was attempted.

Aureomycin hydrochloride* in capsules containing 250 mg. was administered orally to all patients, usually in doses of from 2.0 to 4.0 gm. daily. No toxic side reactions were encountered other than occasional nausea, vomiting and diarrhea which in one case were sufficiently severe to require cessation of treatment.

CASE REPORTS

CASE I. M. O., a thirty-six year old colored female, was admitted to the Presbyterian Hospital on February 15, 1949, with complaints of headache, fever and malaise. Her past history was irrelevant. Three days before admission the patient had a stabbing, bitemporal headache which recurred at intervals of one to two hours. She also noted feverish sensations with an oral temperature rising to 102°F. each night and occasional chilliness, but no true chills. The headache became increasingly severe and rather marked photophobia developed, with pain on deviation of gaze. Pain and stiffness of the neck appeared, with pain along the spine from the mid-thoracic to the occipital region on flexing the head toward the chest. There were no signs of a preceding upper respiratory infection, but two days before admission her throat became slightly sore. At this time she also noticed a tender lump in the right axilla. No skin eruption was observed by the patient prior to entering the hospital. On admission the temperature was 101.6°F. There was a questionable rash consisting of a few small erythematous macules scattered over the face, trunk and arms. The neck was moderately stiff on anterior flexion but the Kernig and Brudzinski signs were absent and there were no focal neurologic signs. The lymph nodes in the right axilla were enlarged and tender but careful examination of the cutaneous area draining to this site failed to reveal a primary lesion. The heart and lungs were negative, as was the abdomen. The spleen could not be palpated. The leukocyte count was 4,440 with a normal differential. The erythrocyte sedimentation rate was 35 mm. in one hour by the Westergren method. A lumbar puncture was done and clear spinal fluid was obtained under normal pressure. Examination of the fluid showed 2 lymphocytes per cu. mm., protein 61 mg. per cent, sugar 65 mg. per cent and negative Wassermann and colloidal gold tests. During the first twenty-four hours following admission the symptoms remained essentially unchanged and the temperature fluctuated between 101° and 101.6°F. Meanwhile, a definite cutaneous eruption appeared which consisted of small erythematous maculopapular lesions scattered sparsely over the face, trunk and extremities. None of these showed vesiculation. One small erythematous lesion was also seen on the right side of the palate. Treatment was then started with aureomycin, 1.0 gm. initially and 1.0 gm. every six hours thereafter for a total of sixteen doses. Within eighteen hours the temperature fell to normal, the headache and other symptoms

* This drug was kindly supplied by the Lederle Laboratories Division, American Cyanamid Company.

disappeared and there was a marked increase in the sense of well-being. The rash faded completely in forty-eight hours. Convalescence was uneventful and the patient was discharged from the hospital on February 22, 1949. The rickettsialpox complement fixation test was negative

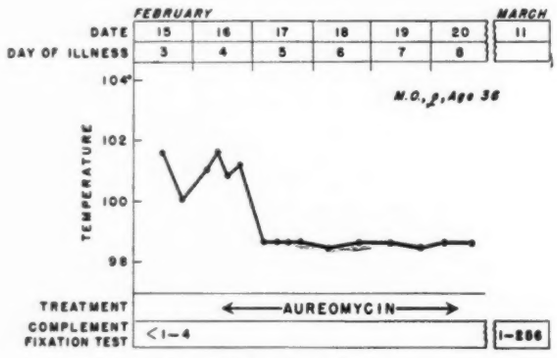

FIG. 1. Chart of Case I.

on the third day of illness but was positive in a serum dilution of 1:256 twenty-four days later. (Fig. 1.)

CASE II. J. B., a thirty-four year old white male, was admitted to the Harkness Pavilion on March 1, 1949. That morning he noted malaise, frontal headache and chilly sensations. These symptoms increased in intensity during the day and his temperature rose rapidly to 102.4°F. between 6:00 and 10:00 P.M., at which time he entered the hospital. Physical examination disclosed nothing of note except a few erythematous papules on the arms, chest and abdomen. The leukocyte count was 7,200 with a normal differential. During the night and up to noon of the following day the patient became more acutely ill with severe frontal headache, generalized muscular aching and repeated chilly sensations. His temperature fluctuated between 101.4° and 103.2°F. An extensive rash appeared over the face, trunk and extremities consisting of erythematous maculopapular lesions, many of which showed vesiculation. There was no enanthem and no primary lesion could be identified. Approximately fifteen hours after admission the patient received 1.0 gm. of aureomycin, 1.0 gm. three hours later and then 1.0 gm. every six hours for a total of ten doses. The drug then had to be discontinued because of nausea and diarrhea. Within the first eighteen hours of treatment the patient felt markedly better, the headache and muscular aching disappeared and the temperature fell to normal. The cutaneous eruption cleared rapidly. Convalescence was uneventful and he was discharged from the hospital on March 5, 1949. The rickettsialpox complement fixation test was negative on the second day of illness and was also negative with serum specimens collected twenty, thirty-three and forty-five days later. R. akari

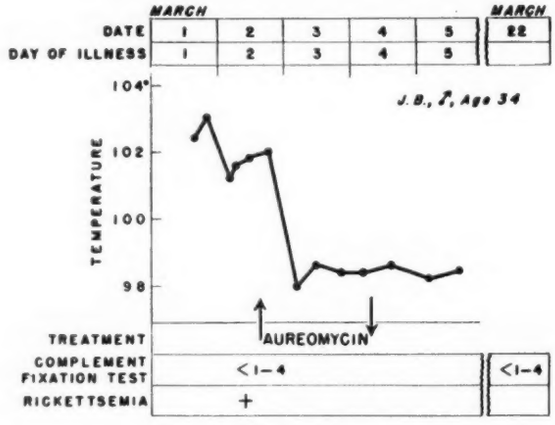

FIG. 2. Chart of Case II.

was recovered from blood obtained on the second day of illness and stored in the frozen state for twenty-two days before isolation was attempted. (Fig. 2.)

CASE III. W. A., a fifteen year old colored male, was admitted to the Presbyterian Hospital on March 25, 1949. The patient apparently had been well until three days earlier when he first noticed a rash on his face and body. Shortly thereafter he had a shaking chill, his temperature rose to 102°F. and a severe frontal headache developed. Fever, chilly sensations, headache and malaise continued until the time of admission. On entrance to the hospital he appeared moderately ill and the temperature was 100.4°F. Scattered over the face, trunk and extremities were many discrete papulovesicular lesions from 3 to 5 mm. in diameter. One vesicular lesion was seen on the soft palate. There was no local lymphadenopathy and no primary lesion could be discovered. The heart, lungs and abdomen were negative. The leukocyte count was 4,100 with polymorphonuclear neutrophiles 27 per cent, lymphocytes 66 per cent, monocytes 4 per cent and eosinophiles 3 per cent. Treatment with aureomycin was started three hours after admission with 1.0 gm. initially, 1.0 gm. after three hours and 1.0 gm. every six hours thereafter for a total of twelve doses. During the first eight hours of therapy the temperature mounted from 100.4° to 101.8°F.; it then fell rapidly to normal. The headache and malaise

disappeared overnight and the extensive cutaneous eruption had virtually disappeared by the end of the third hospital day. Convalescence proceeded without incident and the patient was discharged from the hospital on April 1, 1949. The rickettsialpox complement fixation test

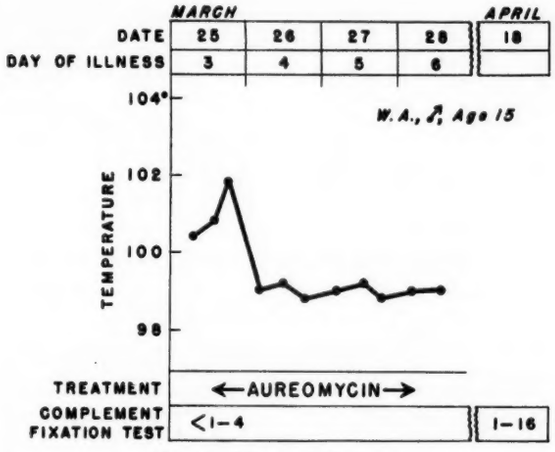

FIG. 3. Chart of Case III.

was negative on the third day of illness but was positive in a serum dilution of 1:16 twenty-four days later. (Fig. 3.)

CASE IV. J. M., a six year old colored female, was admitted to the Babies Hospital on April 8, 1949. Three days before admission the mother noted a small red papule on the right forearm of the patient which she thought was "a mosquito bite." The following day similar papules appeared on the neck, forehead and left buttock. The rash extended further on the day before admission and the child complained of headache, but she did not appear ill and attended school as usual. On the morning of the day of admission the headache became more severe and in the afternoon she had a shaking chill lasting nearly one hour. At the time of entrance to the hospital she appeared acutely ill and her temperature was 103°F. In the right antecubital fossa there was an erythematous lesion about 1.5 cm. in diameter with a central dark crust. The right axillary lymph nodes were enlarged and tender. Many erythematous papules from 2 to 3 mm. in diameter were distributed over the neck, arms, chest and buttocks; some of these showed small dark centers but no vesicles were observed. One lesion was seen on the anterior pillar of the right tonsil. The heart and lungs were negative. The abdomen was negative with the exception that the spleen could be readily palpated. The leukocyte count was 4,500 with a normal differential. Two hours after admission the patient was given an initial dose of 0.75 gm. of aureomycin followed by 0.75 gm. after three hours, 0.5 gm. after another three hours and then 0.5 gm. every six hours until 7.5 gm. had been administered. Within twenty-

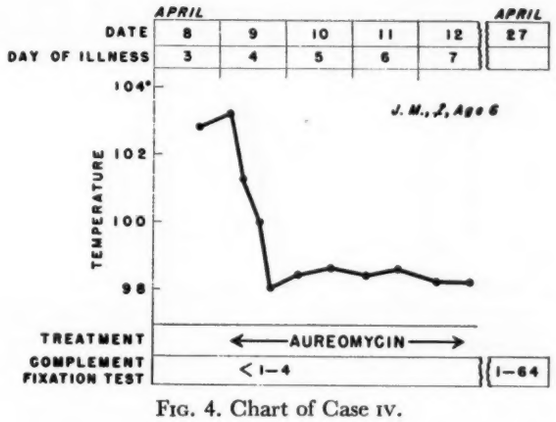

FIG. 4. Chart of Case IV.

four hours the temperature fell to normal and there was marked clinical improvement with complete disappearance of headache. The rash cleared rapidly and convalescence was uneventful. She was discharged from the hospital on April 12, 1949. The rickettsialpox complement fixation test was negative on the fourth day of illness but was positive in a serum dilution of 1:64 eighteen days later. (Fig. 4.)

CASE V. D. R., a twenty-six year old colored male, was admitted to the Presbyterian Hospital on April 8, 1949. On the day before admission the patient had malaise, severe frontal headache and a sensation of fever. He also had several episodes of chilliness followed by profuse sweating but no true chills. On entrance to the hospital about twenty-four hours after onset he appeared acutely ill and had a temperature of 102.8°F. The chief complaint was severe throbbing headache. The conjunctivas were intensely injected and there was considerable pain on deviation of gaze. The heart, lungs and abdomen were negative. No rash was observed. The leukocyte count was 6,000 with a normal differential. During the next twenty-four hours the temperature ranged between 101° and 104°F. Further examination disclosed an erythematous indurated lesion about 1.5 cm. in diameter, with a small central eschar, just above the right elbow posteriorly. The lymph nodes in the right axilla were slightly enlarged and tender. Careful search disclosed an almost inapparent rash consisting of erythematous macules from 2 to 3 mm. in

diameter widely spaced over the arms, trunk and thighs. Some of these lesions exhibited a tiny central vesicle. This eruption would certainly have been missed had it not been deliberately looked for. Meanwhile, although the temperature had dropped somewhat, the patient

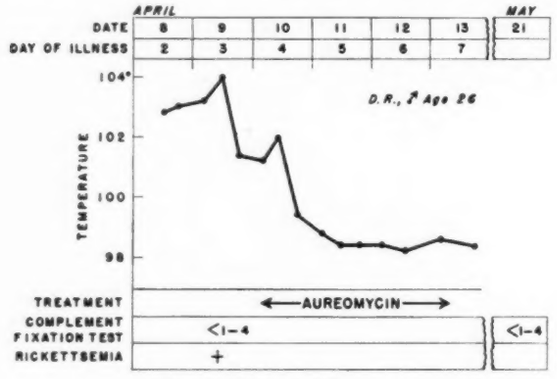

FIG. 5. Chart of Case v.

complained of increased malaise and headache and had some pain and stiffness of the neck. There were no other signs of meningeal irritation and lumbar puncture yielded clear spinal fluid under normal pressure with normal cytologic and chemical findings. Treatment was now started with 1.0 gm. of aureomycin initially, 1.0 gm. after three hours, 1.0 gm. after another three hours and then 1.0 gm. every six hours for a total of fourteen doses. In less than twenty-four hours there was marked clinical improvement with complete disappearance of headache and malaise and a return of the temperature to the normal range. Convalescence was not remarkable and the patient was discharged from the hospital on April 14, 1949. The rickettsialpox complement fixation test was negative on the third day of the illness and was again negative forty-two days later. R. akari was recovered from blood obtained on the third day of illness and stored in the frozen state for fifty-seven days before isolation was attempted. (Fig. 5.)

CASE VI. M. C., a thirty-two year old colored female, was admitted to the Presbyterian Hospital on June 20, 1949. Seven days before admission she noted a small red spot on the left side of her neck which was neither painful nor tender. About three days later she observed a swollen area just above this spot which increased progressively in size. On the day before admission she first felt feverish and had a severe, constant occipital and retro-orbital headache. When admitted to the hospital the temperature was 102.2°F., but the patient did not appear actuely ill. On the lower aspect of the left side of the neck there was an erythematous papule about 1.0 cm. in diameter, with a central dark crust, and just above this was an enlarged, tender cervical lymph node about 2 by 2 cm. in diameter. No other cutaneous lesions were seen and there was

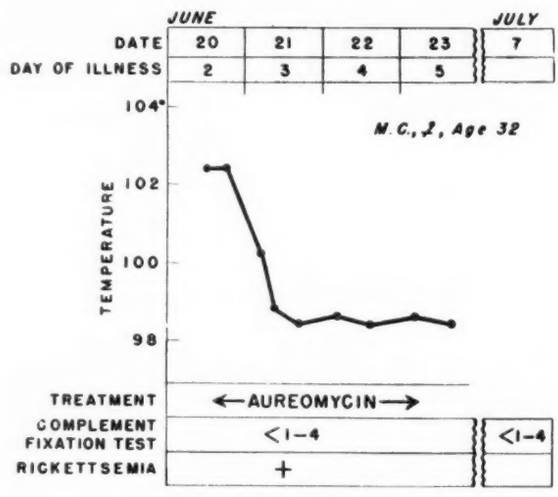

FIG. 6. Chart of Case VI.

no enanthem. The conjunctivas were moderately injected. The heart, lungs and abdomen were negative. The leukocyte count was 4,120 with a normal differential. On account of the severity of the headache a lumbar puncture was performed and clear spinal fluid was obtained under normal pressure. Examination of the fluid showed 4 lymphocytes per cu. mm., protein 60 mg. per cent, sugar 62 mg. per cent and negative Wassermann and colloidal gold tests. One hour after admission she was given 1.0 gm. of aureomycin and then 0.5 gm. every six hours until 5.5 gm. had been administered. The temperature descended to normal within twenty-four hours, the headache rapidly subsided and she was essentially asymptomatic after the first day of treatment. On the third hospital day the leukocyte count was 2,240 with a relative lymphocytosis. At no time was a secondary cutaneous eruption observed. She was discharged from the hospital on June 23, 1949. The rickettsialpox complement fixation test was negative on the first hospital day and was again negative seventeen days later. However, R. akari was recovered from blood collected on the day of admission and stored in the frozen state for 169 days before isolation was attempted. (Fig. 6.)

CASE VII. J. S., a five and a half year old white male, was admitted to the Babies Hospital

on June 22, 1949. About one week earlier the mother noted a small red area on the patient's chin which she thought was an insect bite. Four days before admission the child began to complain of feeling ill with headache and loss of appetite and his temperature was found to be 103°F. The following day a generalized cutaneous eruption appeared. Fever and headache continued together with marked anorexia and on the morning of the day of admission he vomited twice. On entrance to the hospital the patient did not appear acutely ill and the temperature was 102.2°F. Sparsely scattered over the face, trunk and extremities were small erythematous maculopapular lesions some of which showed central vesicles containing cloudy fluid. Two vesicular lesions were seen on the soft palate. Under the chin there was a small erythematous papule with a central dark crust. There was no local lymphadenopathy. The heart, lungs and abdomen were negative. The leukocyte count was 4,000 with a normal differential. On the second hospital day the child still felt ill, the headache was undiminished and the temperature, after an initial fall, had risen again to 102.4°F. Aureomycin was then started in a dose of 0.25 gm. every six hours and was continued until a total dose of 2.25 gm. had been given. Within twenty-four hours the temperature fell to normal, the headache disappeared and the patient appeared markedly improved. Thereafter, he remained well and the rash faded rapidly. He was discharged from the hospital on June 26, 1949. The rickettsialpox complement fixation test was negative on the second day in the hospital but was positive in a serum dilution of 1:16 thirty-four days later. (Fig. 7.)

Case VIII.* K. W., a twenty-two year old white female, was admitted to the Harkness Pavilion on July 11, 1949, with complaints of fever, malaise and glandular swelling of one day's duration. The past history was essentially negative except for a questionable attack of infectious mononucleosis approximately three months earlier. At that time she had had an illness lasting one week characterized by enlargement of the submaxillary and epitrochlear lymph nodes and a slightly enlarged spleen, but no fever. About eighteen hours before admission the patient had marked malaise, pain in the eyes, severe frontal headache and noticed that the lymph nodes in her neck began to swell. During the next few hours the swelling of the nodes increased rapidly and tenderness developed on the right side of the neck. Four hours before admission she felt feverish and found her temperature to be 101.8°F. On entrance to the hospital the patient appeared moderately ill and the temperature was 101.4°F. No skin eruption

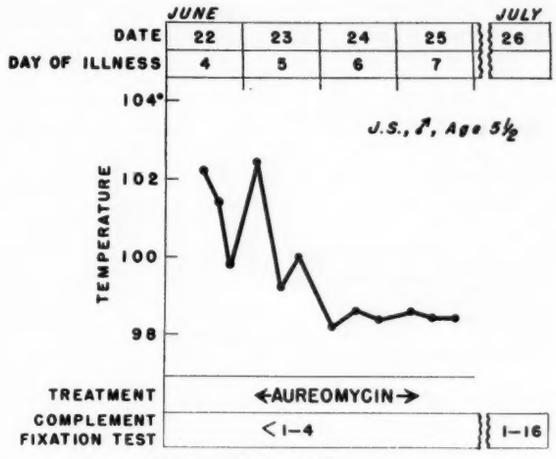

Fig. 7. Chart of Case VII.

was observed with the exception of a single erythematous papule about 0.5 cm. in diameter with a central dark crust which was situated on the right cheek. There was considerable pain in the eyes, especially on movement, but no conjunctivitis. The upper cervical lymph nodes were bilaterally enlarged, especially on the right side where there was marked tenderness. The remainder of the physical examination was essentially negative. The leukocyte count was 6,500 with a normal differential. During the next two days she was treated with 500,000 units of penicillin twice daily with no improvement. Indeed, during this period the temperature rose progressively to 103.2°F. and the patient became more acutely ill with severe headache, marked muscular aching, sore throat and pain in the right cervical area. A generalized cutaneous eruption also appeared which consisted of maculopapular lesions, some with central vesicles, rather widely spaced over the face, trunk and extremities. One similar lesion was observed on the anterior pillar of the right tonsil. Penicillin was now discontinued and aureomycin was given in a dose of 1.0 gm. initially, followed by 0.5 gm. every six hours until 7.5 gm. had been administered. Within twelve hours there was marked symptomatic improvement and at the end of twenty-four hours the temperature had fallen to normal. Thereafter, the patient felt well and the rash and lymphadenopathy disappeared in about

* Case of Dr. F. R. Bailey.

three days. She was discharged from the hospital on July 18, 1949. The rickettsialpox complement fixation test was negative on the first day in the hospital but was positive in a serum dilution of 1:32 fifteen days later. (Fig. 8.)

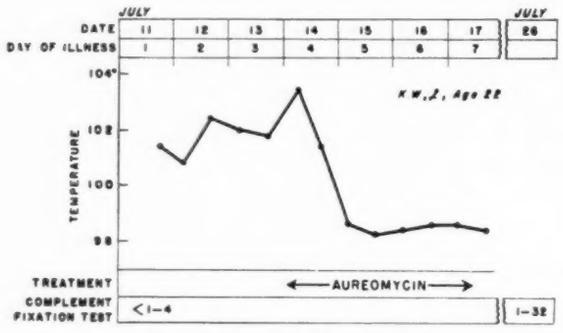

FIG. 8. Chart of Case VIII.

COMMENTS

In our experience, as well as that of Greenberg and his associates,[18,19] the acute febrile stage of rickettsialpox usually lasts for about one week, the fever and associated symptoms abating gradually over the final two or three days of the illness. Only rarely do the acute manifestations of the disease subside as soon or as quickly as they did in each of the patients following treatment with aureomycin. It seems reasonable to conclude, therefore, that the uniformly rapid and favorable therapeutic response was not a chance observation even though the number of cases treated was small, and that rickettsialpox probably may be added to the present list of rickettsial infections in which aureomycin has a prompt curative effect. Rickettsialpox is not a serious or a fatal disease but it incapacitates the patient and often renders him severely ill for several days. The therapeutic use of aureomycin should consequently be considered whenever the clinical diagnosis appears to be reasonably certain.

Another antibiotic with known antirickettsial properties, chloramphenicol, is probably of value in the treatment of rickettsialpox, although reports of its use in the human infection have not been published up to the time of this writing. We have treated three patients with chloramphenicol, all of whom showed a rapid recovery similar to that observed with aureomycin. However, the data are obviously too limited to permit a comparison of therapeutic effects with the two antibiotics. Recently a new antibiotic, terramycin, has been found to have antirickettsial properties,[20] including activity against strains of R. akari in chick embryos.[21] Reports of the use of terramycin in rickettsialpox and other rickettsial infections of man doubtless will be forthcoming in the near future.

Attention should be drawn to the fact that in three of the eight patients treated with aureomycin no specific antibody response was detected during convalescence by the complement fixation test. Nevertheless, there can be no question that these three patients did suffer from rickettsialpox, as evidenced by a characteristic clinical picture and the recovery of rickettsiae from blood taken in the acute phase of the illness. This observation appears to be significant in view of the fact that in more than forty untreated cases of rickettsialpox we have invariably found significantly elevated titers of antibody during convalescence by the same serologic method, using antigen prepared from the same strain of R. akari. It also appears unlikely that the failure to demonstrate an immunologic response in these three patients resulted from a deficiency in their capacity to form antibodies, from wide antigenic differences between the strains of rickettsiae causing their infection and the strain used in the complement fixation test or from factors in the test itself, such as poor sensitivity and errors in technic. It would seem more probable that aureomycin was the responsible factor and that the antibiotic may have suppressed rickettsial multiplication so promptly and completely that the resulting antigenic stimulus was insufficient for detectable antibody production. This hypothesis is supported by earlier observations of Wong and Cox[1] and of Anigstein et al.,[2] who noted that animals infected with typhus and spotted fever rickettsiae were occasionally susceptible to subsequent challenge inoculation when the

initial infections had been treated very early with aureomycin.

If early treatment with aureomycin may occasionally cause suppression or diminution of the immune response following rickettsialpox, it seems probable that a similar phenomenon should occur in other types of rickettsial infections. Information on this point is meager at present but there is some evidence that aureomycin therapy may depress antibody formation following Q fever although complete suppression has not been noted.[22] Since the specific diagnosis of rickettsial disease is usually made in retrospect by serologic tests with acute and convalescent phase serums, it is important to note that any factor which interferes with the immune response may seriously affect the diagnosis by serologic methods and may even render it impossible. The modifying effect of antibiotic therapy on the clinical pattern has also to be considered from the diagnostic standpoint. This is illustrated by Case VI of this series in which early treatment with aureomycin may have been responsible for failure of the secondary cutaneous eruption to appear.

SUMMARY

A prompt therapeutic response was observed in eight cases of rickettsialpox treated with aureomycin. The results suggest that treatment with this antibiotic should be considered whenever the clinical diagnosis of rickettsialpox seems reasonably certain.

In three of the eight patients a detectable, specific, immune response following recovery, as determined by the rickettsialpox complement fixation test, failed to develop. The significance of this finding is discussed.

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Further Experience in the Treatment of Rocky Mountain Spotted Fever with Chloramphenicol*

ROBERT T. PARKER, M.D., ROBERT E. BAUER, M.D., LEONARD M. LISTER, THEODORE E. WOODWARD, M.D. *and* HOWARD E. HALL, M.D.

Baltimore, Maryland

THE original studies on chloramphenicol (chloromycetin®) in experimentally induced infections demonstrated its efficacy as an antirickettsial agent.[1,2] Clinical studies on scrub,[3] murine[4] and epidemic typhus[4,5] treated with chloramphenicol confirmed early impressions and led Pincoffs et al.[6] to investigate the effectiveness of this antibiotic in the therapy of Rocky Mountain spotted fever. The following report presents further affirmative evidence that chloramphenicol is beneficial in the treatment of this rickettsiosis.

METHOD OF STUDY

Selection of Cases. From May to September, 1949, sixteen patients with clinical diagnoses of Rocky Mountain spotted fever were observed. Of these patients thirteen were males and three were females; fourteen were white and two were Negroes. Nine of the patients were twenty years of age or less; five ranged from forty-four to fifty years of age.

Clinical Diagnostic Criteria. The clinical diagnostic criteria adopted for this study were identical with those established by Pincoffs et al.[6] Eleven patients recalled having removed an attached tick during the two-week period prior to their acute illness. The remaining five patients had been exposed to ticks during a similar period of time. All sixteen patients gave a history of persistent daily fever from onset of their acute illness until hospitalization. Furthermore, all patients showed three or more of the following secondary clinical features: photophobia, prominence of headache, tarsal conjunctivitis, periorbital edema, partial deafness, delirium, splenomegaly and mental dullness. In addition, all patients had a rash distinctive in character and in distribution of lesions. Each patient was studied for the presence of other infectious diseases but none was found.

In all patients, the temperature was recorded at four-hour intervals day and night. Temperatures were considered normal when a persistent oral temperature of 99°F. or below was reached or when rectal temperature did not exceed 100°F.

Laboratory Diagnostic Tests. Likewise, the aforementioned authors'[6] laboratory diagnostic criteria were adopted for this study. Prior to the start of specific therapy, guinea pig isolation was attempted on fourteen patients. When inoculated animals developed fever (temperature of 104°F. or higher for three consecutive days) and complement-fixing antibodies (titer of 1:16 or higher) transmission of the disease was considered successful. Positive isolation was obtained in ten cases. Positive agglutination for Proteus OX19 (titer of 1:160 or higher) was found in fourteen patients. All patients' sera were tested for complement-fixing antibodies.† In eleven patients results were considered positive (titer of 1:10 or higher).

The demonstration of a strongly positive reaction in any one of the previously mentioned laboratory diagnostic procedures, that is, animal isolation, Proteus OX19 agglutination or

† We wish to thank the Department of Virus and Rickettsial Diseases, Army Medical Center, Washington, D.C., and the Maryland State Department of Health for performing some of these serologic tests.

† From the Section of Infectious Diseases, Department of Medicine, School of Medicine, University of Maryland, Baltimore, Md. This study was supported by a grant from Parke, Davis and Company, Detroit, Michigan, who also furnished the drug.

complement-fixation, was accepted as confirmation of clinical diagnosis. Of sixteen cases comprising this series three were positive in one test only,* seven were positive in two tests* and six were positive by all three tests. (Table 1.)

TABLE 1

TABULATION OF LABORATORY AND THERAPEUTIC RESULTS IN SIXTEEN CASES OF ROCKY MOUNTAIN SPOTTED FEVER

Patient No.	Age	Sex	Isolation of Rickettsiae	Maximum Proteus OX19 Agglutination	Complement Fixation	Day of Disease Treatment Begun	Days of Treatment	Total Chloramphenicol (gm.)	Duration of Fever after Start of Treatment
1	3	M	0	1:160	Positive	5	3	5.5	2.8
2	8	M	Positive	1:1280	0	4	1	2.0	7
3	5	M	Positive	1:640	Positive	7	6	10.5	6.5
4	50	M	0	1:1280	Positive	5	3.6	16.0	3.5
5	44	M	Positive	1:1280	Positive	7	1.8	7.5	2.5
6	49	M	Positive	1:1280	Positive	8	25	43.25	13
7	39	M	Positive	0	0	4	2.8	10	4.8
8	6	F	Not attempted	1:640	Positive	11	6.5	11.25	2
9	13	M	0	0	Positive	7	1	3.0	1
10	14	M	0	1:320	Positive	6	1	3.0	7
11	13	F	Positive	1:160	Positive	8	6.8	20.75	5.5
12	6	M	Not attempted	1:640	0	9	4.5	9.0	6
13	49	F	Positive	1:160	Positive	8	3	9	6
14	23	M	Positive	1:1280	0	8	3	9	6.5
15	15	M	Positive	1:320	Positive	5	3	9	2.5
16	47	M	Positive	1:640	0	8	6.5	12.5	2.3

CHLORAMPHENICOL THERAPY

Chloramphenicol (chloromycetin®) was dispensed as 250 mg. Kapseals. Therapy was limited to the oral route. Two patients required administration of drug by gavage. Neither nausea nor vomiting was encountered. Patients 6 and 11, who required prolonged administration of the drug, developed glossitis which disappeared after cessation of chloramphenicol treatment. One patient developed a mild urticaria which disappeared when specific therapy was terminated. No other toxic symptoms were noted.

Since the optimal time-dosage schedule for chloramphenicol therapy of this rickettsiosis is not yet known, various schedules were employed. In general, approximately 35 to 75 mg./kg. of body weight were given as an initial loading dose (from 2–3 gm. for adults, and from 0.75–2 gm. for children). Continuous doses given every four to eight hours varied from 0.5–1.0 gm. for adults and from 0.25–0.75 gm. for children. Three patients received a single dose of 2, 3 and 3 gm., respectively. Three patients received discontinuous therapy in the form of a single daily dose of 3 gm. for three days.

On an average, therapy was started on the sixth day of disease. Patients on an initial and continuous dose schedule received an average of 11.4 gm. of chloramphenicol. In nine patients so treated the average duration of therapy was 4.6 days.

Knowledge of chloramphenicol blood levels was not necessary for administration of the antibiotic. Although these determinations were made in the majority of cases, concentrations were not known until therapy had been terminated.

Clinical Response to Chloramphenicol Therapy. The previously noted striking defervescence in Rocky Mountain spotted fever treated with chloramphenicol was unquestionably confirmed in this series. The average dura-

* Isolation was not attempted on one of these patients.

tion of fever after the first dose of antibiotic was 4.4 days.* This value is somewhat greater than the 2.2 days reported earlier from our clinic.[6] With the realization that it is hazardous to compare the degree of effectiveness of a chemotherapeutic agent in two series of patients observed in different years, the variation in average febrile period after the beginning of therapy may be partially explained by the increased number of severe infections and by the greater proportion of older patients in the present series.

At the end of the first treatment day alleviation of symptoms was apparent except for Patient 6. Four patients experienced relief from their severe, frontal headache two to six hours after receiving their first dose of chloramphenicol. In most cases the eruption had receded noticeably after two days of treatment but in four critically ill patients complete disappearance of the eruption did not occur until seven to ten days after therapy had been started. Except for Patient 6, follow-up examinations two to five weeks after discharge revealed no residua.

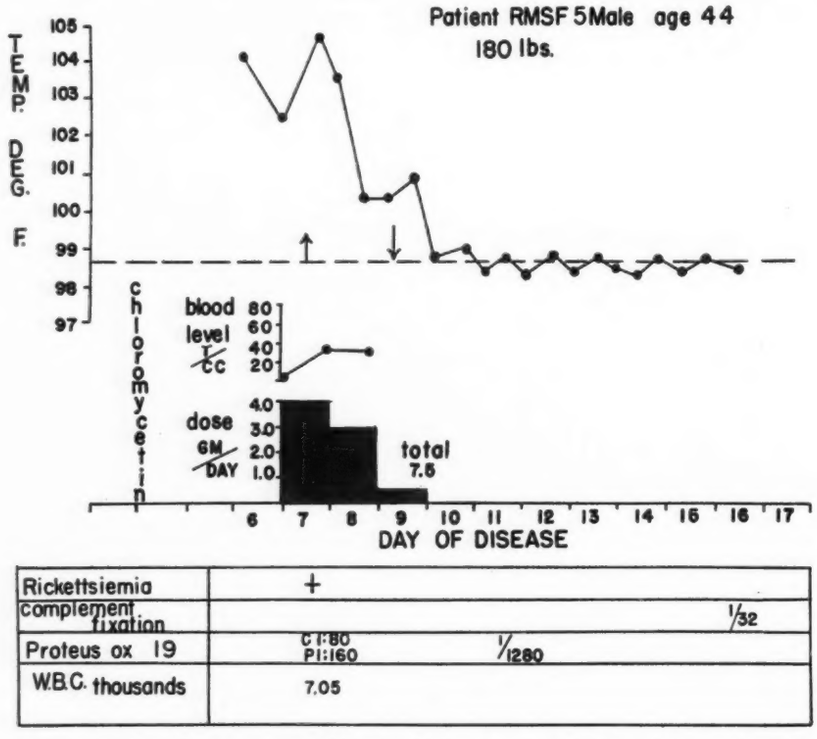


FIG. 1. Patient 5; the course of Rocky Mountain spotted fever in a moderately ill patient who was treated with chloramphenicol.

CASE 5. *Typical Response of a Moderately Ill Patient Receiving Continuous Therapy:* This forty-four year old farmer was admitted on the sixth day of disease with a history of prostrating frontal headache, fever of 102°F., malaise, weakness and rash. Admission physical examination revealed injected conjunctivae, tenderness over the frontal region and a non-fixed macular rash over the entire body except for the hands, face and neck. His cough was productive of bright red blood in small amounts. On the seventh day of disease chloramphenicol was started, 3 gm. initially and 0.5 gm. every four hours. Three hours following the first dose his headache disappeared. Two days later, except for a few petechial lesions, the rash had faded. At that time urticaria was first noted and medication was stopped, a total of 7.5 gm. having been given.

* Patient 6 was not included in the computation of these average values. Because of a pyogenic osteomyelitis which developed adjacent to an area of extreme gangrene over the right tibia, this patient had a prolonged febrile course and received large quantities of chloramphenicol and 21 gm. of aureomycin. It was believed that inclusion of his case in the computation would unduly weigh the average values.

Two days later the urticaria was gone. On both discharge and follow-up examinations the patient was asymptomatic. The significant clinical and laboratory findings are graphically presented in Figure 1.

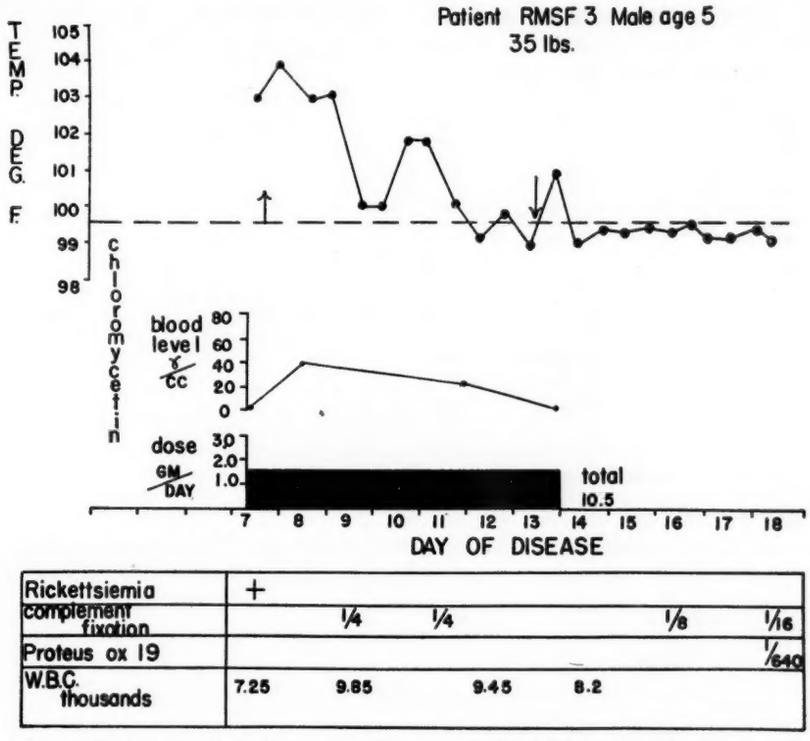


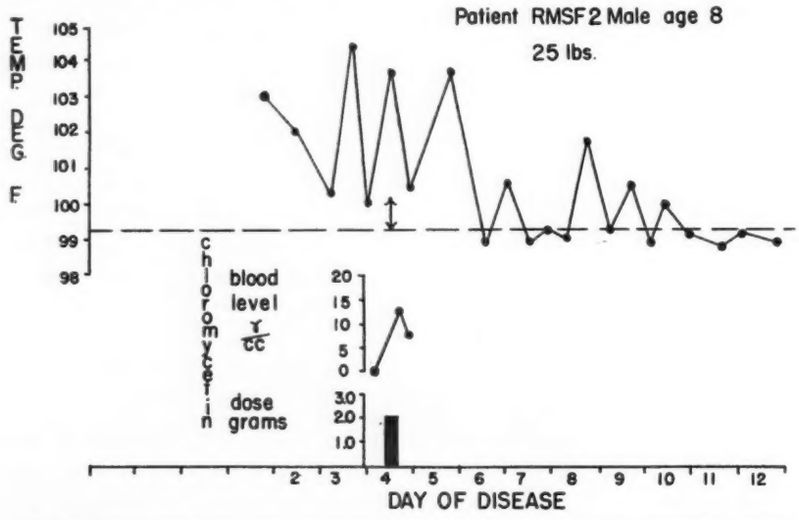
FIG. 2. Patient 3; clinical response of a severely ill child who received energetic supportive therapy as well as chloramphenicol; convalescence was uncomplicated.

CASE 3. *Course of Disease in a Critically Ill Patient:* Patient 3, a five year old boy, was admitted on the seventh day of disease with a history of persistent fever of 104°F., posterior cervical and anterior chest pain and rash on the arms and legs. During the two days prior to admission he was unable to sit up. Admission physical examination revealed an irritable, semidelirious child with edema of the face, hands and feet. There was a pink, hemorrhagic rash over the entire body including the palms and soles. Immediately, chloramphenicol therapy was started, 1.5 gm. initially and 250 mg. every four hours. During the first several days of hospitalization supportive therapy included transfusions of whole blood, plasma, saline and glucose infusions, competent nursing care and a liquid diet containing large quantities of protein. After the first day of therapy there was slight improvement. On the third day of treatment, although edema of the face remained, the rash was petechial in character and the patient was considerably more alert. Four days later he was mentally alert, able to sit up with assistance and eating well. Convalescence was uneventful. The patient's clinical course is graphically presented in Figure 2.

Course of Disease in Patients Receiving Single Doses of Chloramphenicol. Smadel et al.[7] have shown that tsutsugamushi disease (scrub typhus) can be treated with single doses of chloramphenicol. In an effort to observe this effect in Rocky Mountain spotted fever and to obtain additional information pertaining to the minimal curative dose, three patients were treated with single doses (2–3 gm.) of chloramphenicol. Figure 3 illustrates the findings in one of these cases. In two of these patients (Patients 2 and 10, Table 1) symptomatic relief was rapidly obtained even though the febrile period was somewhat prolonged (seven days).

Course of Disease in Patients Receiving Single Daily Doses of Chloramphenicol. Payne, Sharp and Knaudt[5] have shown that epidemic typhus can be treated with single daily

doses of chloramphenicol. In the present series this therapeutic regimen was utilized on a small number of patients with Rocky Mountain spotted fever. Patients 13, 14 and 15 received single daily doses of 3 gm. of chloramphenicol on three consecutive days. Approximately three hours after administration of the drug blood levels ranged from 13.5 to 48 gamma/cc.* Blood levels were not determined at longer periods, i.e., twenty to twenty-four hours after medication had been given. However, it has been shown by previous investigators[10] that twenty-two hours after a 3 gm. oral dose of the drug there is no measurable chloramphenicol remaining in the blood. The duration of fever after beginning of specific medication in these three patients was 6, 6.5 and 2.5 days. After sixty hours of normal temperature Patient 15 had a temperature elevation ranging from 101°F. to 102.8°F. which lasted for forty-four hours. During this febrile period, the patient experienced a return of pre-treatment symptoms, i.e., severe frontal headache, backache, malaise and anorexia. Attempted isolation of rickettsiae from blood taken during this episode was unsuccessful. Without additional therapy, the patient became and remained afebrile and asymptomatic.



Rickettsiemia	+			
complement fixation		0	0	
Proteus ox 19		0		1:1280
W.B.C. thousands	9.0			7.0

FIG. 3. Patient 2; clinical course of Rocky Mountain spotted fever in response to a single dose of chloramphenicol (2 gm.) administered on the fourth day of disease.

Guinea pig inoculation of pre-treatment blood specimens from Patients 13, 14 and 15 was positive (criteria previously described). Twenty-four hours after administration of one 3 gm. dose to two of these patients attempts to isolate rickettsiae were unsuccessful. Likewise, isolation attempts twenty-four hours after the administration of 2 single 3 gm. doses to two patients gave negative results. Chloramphenicol blood levels were not performed on these specimens.

CASE 13. *Course of Disease in a Patient Who Received Discontinuous Therapy:* Patient 13, a forty-nine year old white woman, was admitted on the seventh day of disease with a history of malaise, weakness, fever, headache, tinnitus and rash. On admission the illness was manifested by photophobia, mental confusion, partial deafness, splenomegaly and maculopapular lesions on the

* These determinations were obtained by a modification[8] of the turbidimetric assay method reported by Smith et al.[9]

ankles, thighs and wrists. On the eighth day of illness the patient received a single 3 gm. oral dose of chloramphenicol and subsequent single 3.0 gm. doses for two days. On the second day of therapy the headache had disappeared but the deafness had progressed. The following day there was considerable symptomatic improvement and the rash had become petechial in character. Except for persistence of partial deafness for two weeks the patient became rapidly asymptomatic. The essential findings in this case are graphically presented in Figure 4.

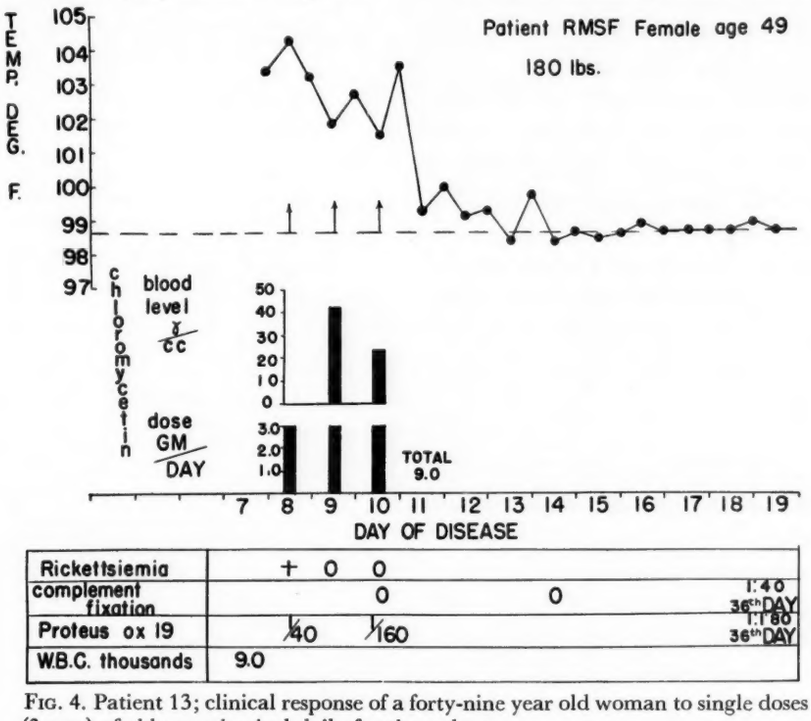


FIG. 4. Patient 13; clinical response of a forty-nine year old woman to single doses (3 gm.) of chloramphenicol daily for three days.

COMMENTS

In view of the small number of available patients, those untreated were not observed during our study. However, a series of eighty-five patients with Rocky Mountain spotted fever treated at the University Hospital, Baltimore, during the pre-specific treatment years from 1930 to 1945 may be used as a basis for comparison. In that series the average febrile course in days was sixteen, with a range from thirteen to twenty-one. The mortality rate was 23.5 per cent. In the present group of sixteen cases after initiation of treatment the average febrile period was 4.4 days and no fatalities occurred. While the number of treated patients is admittedly small, it may be concluded nevertheless that chloramphenicol is highly efficacious in the treatment of Rocky Mountain spotted fever.

In the management of severely ill patients, sole reliance upon administration of this antibiotic is not sufficient. The extensive peripheral vascular damage in these patients makes parenteral supportive therapy (i.e., whole blood, plasma, saline and glucose) essential. Additional supportive measures should include competent nursing care and administration, orally or by gavage, of a high protein diet.

In the group of cases herein reported chloramphenicol was started on about the sixth day of illness, range fourth to eleventh day. Regardless of the stage of disease when specific therapy was instituted, the clinical response was uniformly favorable. The duration of treatment in patients receiving a large initial dose and subsequent continuous doses averaged 4.6 days, range 1.8 to 6.8 days. Despite the demonstrated efficacy of single daily doses of chloramphenicol, this form of therapy is not presently

recommended although the results invite further testing. Until additional data are available it is recommended that patients acutely ill with Rocky Mountain spotted fever receive the following initial dose of chloramphenicol: 0.5 to 1.5 gm. for children and 2 to 3 gm. for adults. The antibiotic should be given at four- to eight-hour intervals with the total daily dose approximately 1.5 gm. for children and 3 gm. for adults. Medication may be discontinued after twenty-four hours of normal temperature.

Chloramphenicol blood level determinations are not essential for following the course of disease in Rocky Mountain spotted fever. Indeed, although these tests were performed on practically all patients, in no instance was the concentration known until convalescence.

SUMMARY

The data on sixteen patients with Rocky Mountain spotted fever treated with chloramphenicol are presented. Findings in these patients confirm previous results that the antibiotic is an effective chemotherapeutic agent for treatment of this rickettsiosis.

Acknowledgment: The authors are indebted to the physicians of this vicinity for their cooperation in referring most of the patients in our study to the University Hospital. Drs. Robert Ensor, Thaddeus Siwinski and Carlos Constellano also kindly granted permission for study of their patients. The technical assistance of Miss Ann Merideth is gratefully acknowledged. Assistance rendered by the house staff of the University Hospital is appreciated.

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Plasmacytosis and Hyperglobulinemia as Manifestations of Hypersensitivity*

A Postmortem Study of Two Cases with Hypersensitivity Probably to Sulfadiazine

THEODORE ROBERTSON, M.D.
New York, New York

THE pathology of hypersensitivity has received much attention during the past few years and several clinical and experimental studies have been reported which indicate that plasmacytosis (plasma cell hyperplasia) and hyperglobulinemia may be associated with hypersensitive states.[1–5] This appeared to be so in the two cases now to be described.

In Case 1 sulfadiazine and penicillin were administered in the usual dosages and under hospital supervision to a young woman who had developed acute peritonitis following self-induced abortion. A drug rash and drug fever appeared and with continued administration of the drugs sulfonamide crystaluria, hematuria and cylindruria were noted. Following this, kidney damage and then liver damage became evident. Although sulfadiazine medication was discontinued, the patient died in kidney failure thirty-five days after she first received treatment. Postmortem examination disclosed a severe interstitial nephritis, acute hepatitis and severe and widespread infiltration of almost all organs by plasma cells. The details of the case follow:

CASE 1. This nineteen year old Negress had attended the New York Hospital outpatient department since the age of ten, when she had pulmonary tuberculosis of the childhood type which healed without incident. At no time were sulfonamide drugs prescribed. During her eighteenth year she suffered a head injury and was unconscious for a period of days at another hospital. In the fall of 1947, at the age of nineteen, she had intercourse on several occasions and thereafter noted swelling of her breasts and absence of menstruation and believed that she was pregnant. On New Year's Eve, 1947, some three months after her last menstrual period, she induced an abortion with a douche nozzle. The day following this manipulation she had severe lower abdominal pain and passed large blood clots from her vagina. Intermittent lower abdominal pain and mild bleeding continued for two weeks; then she became unable to void or defecate and had abdominal pain, chills and fever. On January 16, 1948, she presented herself at the outpatient department and was admitted to the gynecologic service.

Examination revealed an acutely ill woman with fever (39°C.) and tachycardia. The abdomen was diffusely tender and rigid below the umbilicus. The cervix was displaced anteriorly and an inoculation of the purulent discharge from the cervical os grew gonococci on suitable media. A tense, tender, fluctuant mass filled the pelvis. There was slight anemia and the white blood cell count was 32,500. The morning after admission a posterior colpotomy was performed under gas-oxygen-ether anesthesia, the patient meanwhile having been placed on a daily regimen of 5.0 gm. of sodium sulfadiazine administered intravenously in molar lactate infusions and 600,000 units of penicillin given intramuscularly. Three separate abscess cavities were drained by the operation. Cultures of these grew Staphylococcus albus and non-hemolytic aerobic streptococcus. Following this the patient improved markedly although the tachycardia and anemia present on admission continued. By the eighth day she was afebrile. The white blood

* From the Department of Pathology, The New York Hospital-Cornell University Medical Center, New York, N. Y.

count dropped from 32,500 to 15,000 during the first eleven days. Differential counts throughout her illness always showed a good neutrophilic response without abnormal cells. Intravenous sodium sulfadiazine was stopped on the fourth day after admission and she was then given oral sulfadiazine (6.0 gm. daily) and sodium bicarbonate in addition to penicillin (160,000 units daily) intramuscularly, except on the eighth and ninth days when no sulfadiazine was administered.

TABLE 1

CLINICAL COURSE IN CASE 1

Days after admission:	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Blood:																	
Hb. gm. %				11.0			11.5	13.5		10.3	9.8	10.6	10.7	11.0	10.5	11.0	9.3
W.B.C. (000)	6.8			9.8	40.0		19.1	12.0	15.9	21.0	13.8	16.5	20.0	23.2	21.3	34.8	22.6
Protein gm. %, A/G											5.1	2.5		2.1	2.0	1.9	1.8
												1.9		2.2	2.7	3.2	3.1
Icterus index											30	67		48	40	36	33
NPN mg. %											35	33		67	99	116	124
Uric acid mg. %											2.3			5.4	6.3	6.7	8.9
Sulfadiazine level mg. %	11.0										6.6					17.4	
Urine:																	
Sulfa crystals	+	No		+			No		No	No	No	No	No	No	No	No	No
R.B.C.	No	No		+			+		No	No	No	+	+	+	+	+	+
Casts	No	No		+			No		No	+	+	+	+	No	No	+	+
Other											bile		alb.	alb.	alb.	alb.	alb.
Treatment:																	
Streptomycin (gm.)							1.0	X	2.0	X	X	X	X	X			
Penicillin (1000 units)	600	X	160			600		600	X	1200	X	X	X	X	X	X	X
Sulfadiazine (gm.)	6.0 p.o.									5.0 i.v.	X	X	2.5 i.v.	5.0 i.v.			

On the twelfth day after admission pelvic examination under anesthesia revealed patency of the drainage wounds, no fluctuant masses and only adnexal induration. During the next two days she had a moderate temperature elevation and abdominal tenderness, and red blood cells and sulfonamide crystals were found in her urine. On the fifteenth day she had a shaking chill followed by tachycardia and a fever to 41.2°C. Up to this time the blood sulfonamide values had ranged between 7.2 and 9.5 mg. per cent. The fever subsided over the next two days and on the eighteenth day was normal, coincident with the appearance of a fine pruritic rash on her extremities, sulfonamide crystaluria and a blood sulfonamide value of 11.0 mg. per cent. During this episode the white blood cell count fell precipitously from 22,000 to 10,000 and then to 7,000 and the hemoglobin to 9.5 gm. per cent. Sulfadiazine medication was discontinued and the patient received two small transfusions without reaction. However, sulfonamide crystals continued to appear in her urine together with red cells and hyaline casts. The rash subsided only to reappear on the twenty-third day as a diffuse, maculopapular, intensely pruritic eruption over the entire body. On this day also her temperature rose to 40.5°C. and thereafter was elevated until shortly before her death. (Table 1.) Intramuscular streptomycin therapy was instituted on the twenty-fourth day and the patient was placed in an oxygen tent. Chest x-rays were normal, there was no peritoneal tenderness and pelvic examination was unrevealing. Peri-orbital edema appeared on the twenty-sixth day and persisted thereafter. Ascitic fluid accumulated. Sodium sulfadiazine was reintroduced intravenously on the twenty-seventh day. Oliguria, jaundice and biliuria developed. Blood serum proteins were low. (Table 1.) Later laboratory studies revealed uremia and serum globulin values that increased during the last six days of life to a value of 3.2 gm. per cent. Physical examinations and numerous x-rays failed to reveal localized inflammation. Wangensteen drainage and transfusions did not effect relief. The uremia rapidly progressed and severe albuminuria and hematuria continued despite cessation of sulfadiazine medication. Although the temperature fell gradually, tacycardia persisted and a gallop

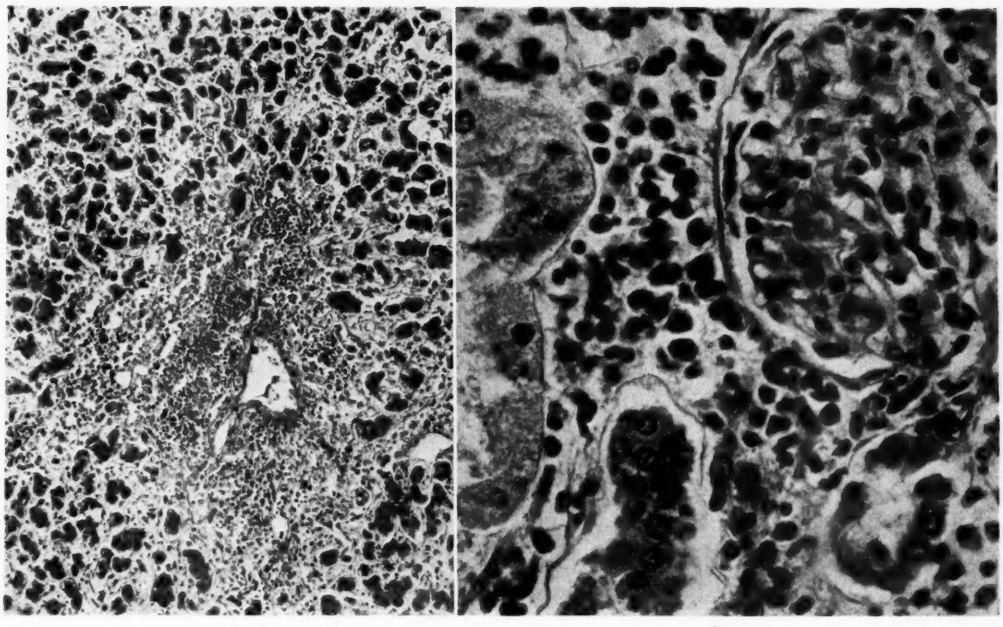

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FIG. 1. Case 1. Acute necrosis of the liver with massive plasmacytic infiltration about a central vein, necrosis of parenchyma cells and partial collapse of the reticulum framework; hematoxylin and eosin stain, approximately 90 ×.

FIG. 2. Case 1. Higher magnification of the kidney lesion. (Fig. 3.) Note the swollen cells in Bowman's capsule and the granularity of the tubular cells; hematoxylin and eosin stain, approximately 450 ×.

rhythm was present on several occasions. Finally, diffuse peripheral edema developed and then signs of bronchopneumonia. On her thirty-fourth hospital day her temperature, which had been almost normal, rose suddenly. She had severe epistaxis, became semi-comatose and expired.

Postmortem examination (autopsy No. 12752 performed ten hours after death) revealed a generalized scaling of the skin most marked on the dorsum of the forearms. There was also severe generalized edema, particularly of the lower extremities. The sclerae were yellow. The abdomen was protuberant, distended by 2,100 cc. of clear yellow ascitic fluid with a specific gravity of 1.017. There were many firm adhesions between loops of bowel in the pelvic cavity and here also were a few small areas of fibrinous exudate over peritoneal surfaces. The uterus was about twice normal size and soft. Both Fallopian tubes were thickened and the ovaries were adherent in the posterior cul-de-sac where there was an abscess 2 cm. across from which about 1 cc. of pus was obtained. Staphylococcus albus was cultured from this material.

There were 300 cc. of yellow fluid in the right pleural cavity and 350 cc. in the left. No thymic tissue was identified; the mediastinal lymph nodes, however, as well as most of the other nodes of the body were enlarged and soft. No pleuritis or pericarditis was found. The heart was small (200 gm.) and appeared normal. The lungs together weighed 800 gm. and a frothy fluid oozed from the cut surface. The spleen was enlarged (250 gm.) and very soft and red. One small white infarct was evident. The liver was enlarged (1,920 gm.), yellowish-brown and very soft, the parenchyma bulging beyond the capsule when the organ was cut. The biliary system was normal as was the gastrointestinal tract. The pancreas and adrenals appeared unremarkable. Each kidney, however, weighed 230 gm. and appeared swollen. The cortices were pale and widened and there were prominent red streaks in the pyramids. The pelves and ureters were unremarkable and the bladder wall was only slightly hemorrhagic and roughened. The bone marrow was red and firm and no skeletal changes were noted. The brain and meninges appeared unremarkable in the gross but two small areas of softening were seen in a section through the pons. Culture of heart's blood taken postmortem was sterile.

Microscopic examinations revealed a severe, generalized dissolution of the liver elements. The hepatic cord cells around each central vein

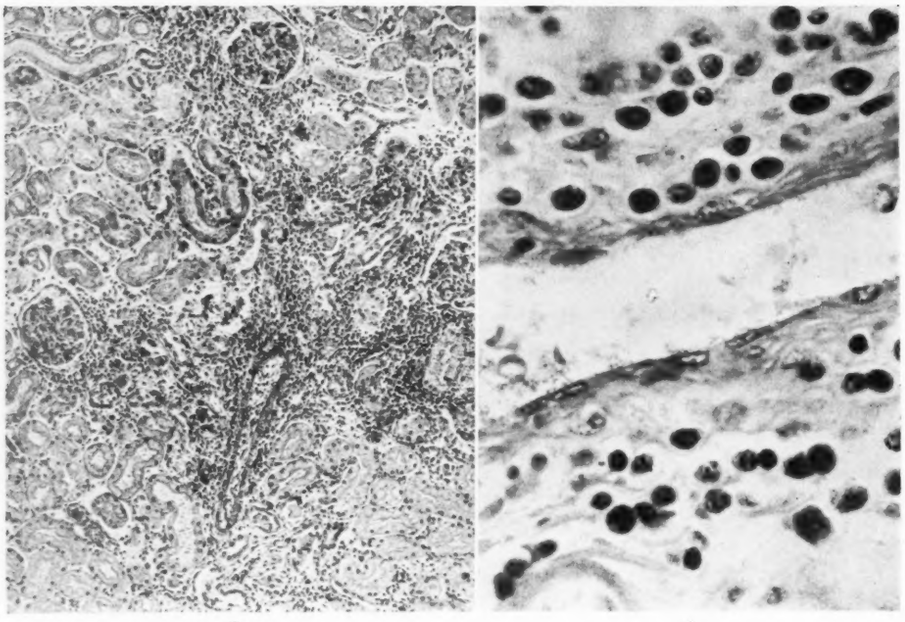

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FIG. 3. Case I. Spread of the plasmacytic infiltration in the kidney out along blood vessels to glomeruli. Note the granularity of the cytoplasm of the tubular epithelium, and the protein precipitate in Bowman's space and in the lumens of the tubules; hematoxylin and eosin stain, approximately 90 ×.

FIG. 4. Case I. Perivascular cuffing in the capsule of a lymph node by cells of the plasmacytic series. Most of the plasma cells are immature and many exhibit nucleoli. This lesion was seen in many organs; erythrosin-azure-methylene blue stain, approximately 700 ×.

were for the most part absent, although a few necrotic cells in various stages of lysis were found. There was only moderate collapse of the reticulum framework. The necrosis of parenchymal cells extended up toward the portal areas in a haphazard and intermittent fashion. (Fig. 1.) No regenerating cells were found. There was inspissated bile in some canaliculae. Plasma cells infiltrated both portal and central areas; this will be more fully described later. No micro-organisms were found in the liver with either the Giemsa or Levaditi stains.

The kidney was diffusely infiltrated by leukocytes, mostly plasma cells. The glomeruli were relatively undamaged although many tufts were swollen and exhibited an increased number of nuclei. An eosinophilic granular material often occupied Bowman's space. (Fig. 2.) The proximal and distal convoluted tubules were eosinophilic and finely vacuolated but not necrotic. The vasculature of the kidney was normal except that in areas of intense interstitial infiltration the arteriolar walls were slightly thickened and granular.

The plasmacytosis in this case was most marked in the liver, kidneys and lymph nodes; it was, however, also prominent in the heart, skin and spleen, and plasma cells were found in almost all of the other tissues. They were collected about blood vessels, suggesting a periarteritis or periphlebitis, but spread into adjacent tissue, notably in the heart and in the kidney where the infiltration extended along blood vessels to and around glomeruli. (Fig. 3.) The cells varied from mature, well differentiated plasma cells through intermediate forms to more or less primitive cells. The mature cells were about 12 micra in longest diameter with eccentric nuclei, the chromatin of which was clumped at the periphery. The cytoplasm was abundant and moderately basophilic with hematoxylin and eosin stain, was sometimes vacuolated and often had a perinuclear, clear area. Binucleate cells of this type were seen infrequently. The less differentiated cells were usually larger with an eccentric vesicular nucleus and a nucleolus. The most primitive cells were also large with a centrally located large vesicular nucleus containing a nucleolus and with basophilic slightly granular cytoplasm. Intermediate stages between these extremes were readily found. Occasional lymphocytes and polymorphonuclear leukocytes were seen in these accumulations but generally were found

in blood vessels where cells of the plasmacytic series were uncommon. Very few actively phagocytosing cells were seen. The perivascular nature of the lesion was striking in the capsule of the lymph nodes (Fig. 4) and adrenals; nowhere was definite necrosis of vessel walls apparent. There was, however, a trabeculitis in the spleen. The collagen network of some of the trabeculae was broken and infiltrated by neutrophilic polymorphonuclear leukocytes.

The Malpighian follicles were largely obscured and there was a slight increase in the fibrous tissue about the arterioles of the follicles. Cells of the plasmacytic series were scattered throughout the organ as were, to a much lesser extent, cells of the myeloid series. The microscopic appearance of the infarct was in no way unusual. In addition to the capsular accumulations about lymph nodes the body of the nodes was diffusely infiltrated with plasmacytic cells. Although the nodes were enlarged, the characteristic follicular appearance of hyperplastic nodes was absent and plasmacytic cells were scattered in the apparent follicular centers, through the cords and in the peripheral sinuses. There were no tumor-like accumulations of plasma cells in the nodes or elsewhere. The vertebral marrow was not particularly hyperplastic but showed active hematopoiesis of all elements including scattered plasma cell formation. Skin from the abdomen showed slight hyperkeratosis with slight plasmacytic perivascular infiltration in the deeper layers. Small focal interstitial collections of mature and immature plasma cells were found in the myocardium and in the epicardial fat adjacent to the myocardium. Occasional myocardial fibers showed dissolution and basophilia. (Fig. 5.) The vertebral bone marrow was moderately hyperplastic and cells of the plasmacytic series were present in addition to normal elements.

There was a small area of decidual reaction in the uterus with surrounding chronic endometritis but no retained placenta. The lungs contained some precipitated protein and scattered hemosiderin-laden macrophages in the alveolar spaces, and slight plasmacytic infiltration of alveolar walls.

The major final diagnoses were: Infected abortion with pelvic abscesses and peritonitis, largely healed. Hypersensitivity reaction, due probably to sulfadiazine, and characterized by plasmacytosis of many viscera, notably kidney, liver, lymph nodes and spleen, interstitial nephritis with uremia, and acute massive necrosis of the liver with jaundice.

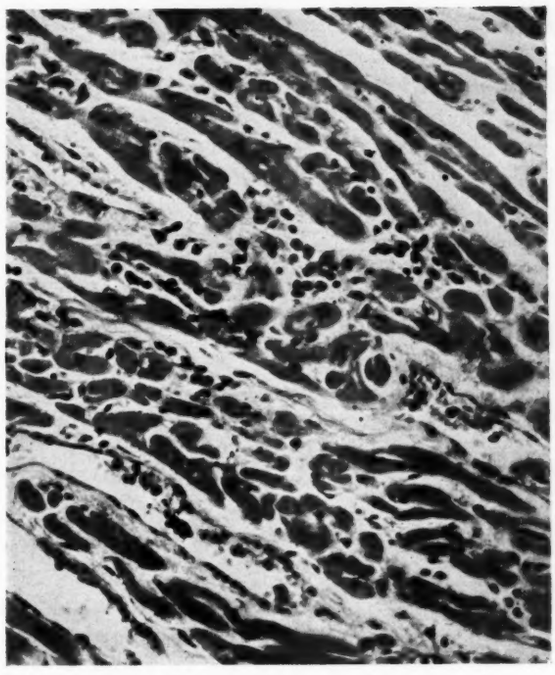

FIG. 5. Case I. Interstitial myocarditis with plasmacytic infiltration and degeneration of individual myocardial fibers; hematoxylin and eosin stain, approximately 240 ×.

Case II was a youth who was treated with sulfadiazine for bronchopneumonia and apparently recovered completely within a week. About a week later, however, fever recurred and with it some back pain and he was hospitalized. Because it was thought that he had active rheumatic fever with subacute bacterial endocarditis, he received massive doses of penicillin for four weeks. Severe anemia, which failed to respond to any therapy, was noticed early in the course, and jaundice and other evidences of liver damage appeared as did a striking hyperglobulinemia. The patient died in liver failure almost six months after the onset of symptoms. Postmortem studies revealed a subacute hepatitis, interstitial nephritis and massive plasmacytic infiltration of many of the viscera similar to that of the preceding case.

CASE II. This fourteen year old Negro boy cut his finger during the Christmas vacation of 1946. The wound became infected and healed

only after a physician treated it with an ointment. On January 12, 1947, the patient was out of school with a "chest cold" which cleared without treatment. He returned to school after four days and resumed strenuous track athletics. Three days later (January 19th), he developed chest pain and cough and his physician made a diagnosis of left bronchopneumonia for which he prescribed oral sulfadiazine. Within forty-eight hours the fever had disappeared and treatment was stopped. On January 23rd the fever recurred and sulfadiazine medication was resumed. The fever subsided within twenty-four hours. It is not known when the sulfonamide medication was stopped. On February 2nd, however, the patient's temperature rose to 102°F.; he had severe pain in the right lower back and hip and was admitted to a local hospital the following day.

On admission his temperature was 102.6°F., pulse 136, respirations 28 and blood pressure 116/64. His heart appeared to be enlarged, with a systolic thrill and murmur at the apex and a questionable presystolic murmur in this area. The liver was palpable. No other significant physical findings were noted.

Laboratory findings (Table II) revealed a severe anemia and no leukocytosis. The urine was normal. Blood cultures taken on that day and on the two succeeding days grew Streptococcus viridans. The sedimentation rate was increased. Roentgenograms of the chest, pelvis and right hip were read as negative. The impression was that the patient had active rheumatic fever, with subacute bacterial endocarditis although there was no evidence of embolic phenomena. Massive penicillin therapy was instituted on the third hospital day and in the following twenty-six days he received 29 million units of penicillin intramuscularly. Apparently he received no further sulfonamide medication for the duration of his life. Blood cultures taken on the sixth hospital day and thereafter showed no growth. His temperature rose to 103°F. on the first three days, then gradually fell to normal by the eighth day and remained so. Early in the penicillin regimen he developed a leukopenia as low as 2,900 white cells per cu. mm. with a depression in all elements. There was also an intermittent eosinophilia as high as 12 per cent. Despite repeated whole blood transfusions he continued to be severely anemic. There is no mention of the condition of the platelets during this period. An electrocardiagram shortly after admission revealed a P-R time of 0.15 and low T waves.

Two weeks after admission the spleen was thought to be enlarged and a sternal marrow aspiration at this time was interpreted as indicating toxic depression of all elements. On a meat-free diet his stools were benzidine-positive.

The prothrombin time was greatly prolonged twenty-four days after admission and within a month liver damage was severe. (Table II.) No sickling of the red blood cells was evident at this time. During his second month of hospitalization two electrocardiograms were interpreted as indicating severe myocarditis.

Since anemia continued despite transfusions and since the spleen remained palpable, the latter was removed on April 16th, seventy-two days after admission. The spleen measured 10 by 15 by 8 cm. and had a fleshy appearance, with prominent follicles. After the operation his temperature rose to 103°F. and thereafter he had a daily elevation above 102°F. for fourteen days. The icterus index rose to 20. X-rays indicated postoperative atelectasis of the right middle lobe and penicillin therapy was re-instituted, although blood cultures were negative. A bone marrow aspiration done a month after splenectomy was considered essentially normal. Evidence of liver damage continued. On June 5th, after four months of hospitalization, the boy was still severely anemic with a reduced number of blood platelets and a normal white count. There was slight albuminuria. At this time he was transferred to the New York Hospital.

On examination at this hospital prominent pale striae were seen in the skin of the shoulders, flank and thigh, although he said he had not lost weight. There were a few petechiae on his right forearm and chest and his breath had a "sweet" odor. The blood pressure was 130/80 and the pulse 100. An apical systolic cardiac murmur was heard. The liver was apparently enlarged and not tender. Neurologic examination was normal. A twenty-four-hour preparation revealed marked sickling of the red blood cells. There was a macrocytic hyperchromic anemia; the white cell count and differential were normal but the platelets were diminished in number. An Osgood-Haskins test revealed a trace of Bence-Jones' protein but the usual heat test was negative. These studies were prompted by the unusual serum protein findings. On three occasions the total serum proteins were found to be markedly elevated. This was due to

TABLE II

SEROLOGIC,* CHEMICAL, BACTERIOLOGIC AND HEMATOLOGIC STUDIES IN CASE II

Date	Hb.	R.B.C.	W.B.C.	Differential	Platelets	Sickling	Liver Function Tests	Serum Protein (gm. %)	Bone Marrow	Culture
2/3/47	8.5	1.86	7,400	Polymorphs 68, lymphocytes 30, monocytes 2						Strep. viridans
2/4										Strep. viridans
2/5										Strep. viridans
2/8										Negative
2/15	Severe anemia		Severe leukopenia to 2,900	Eosinophilia up to 12%	192,000				"Toxic depression"	
2/27–3/1	Severe anemia		Severe leukopenia to 2,900	Eosinophilia up to 12%		Negative	Prothrombin time: Undiluted Diluted Patient 25.5 106 Control 14 33 Icterus index 10			
3/13	Severe anemia		Severe leukopenia to 2,900	Eosinophilia up to 12%	180,000		Cephalin flocculation 4+			
4/2	Severe anemia		Severe leukopenia to 2,900	Eosinophilia up to 12%			B.S.P. 50% retention—30 min. 30% retention—60 min.			
4/19	Severe anemia						Icterus index 20			Negative
5/14	Severe anemia						Prothrombin time: Patient 34 Control 15		Normal	Negative
6/5	9.0	2.3	8,700	Polymorphs 66, lymphocytes 30, monocytes 4	154,000					
6/7	7.0	1.5	9,100	Normal	Diminished					Negative
6/9	7.5	1.6	8,100	Normal (2% eosinophiles)	Diminished	Marked	Prothrombin time: Undiluted Diluted 43 198	12.0 1.3/10.7		Negative
6/10						Marked	Icterus index 12			Negative
6/11–6/12	9.0	2.0	8,800	Eosinophiles 9%	8,000		Icterus index 20, bilirubin 3.0, thymol turbidity 10	12.5 1.3/11.2		
6/17	9.5	2.2					Prothrombin time: Undiluted Diluted 46.5 196.3			
6/20–6/23	9.5		6,000			Marked	B.S.P. 39% retention 45 min. Thymol turbidity 14	11.3 1.8/9.5		
6/26			8,200	Normal			Alk. phosphatase 6.6, total cholesterol 60.0, chol. esters 41.5			E. coli communis
6/27	9.0	2.4	4,900	Normal	Diminished					E. coli communis

* Serologic tests for syphilis were negative.

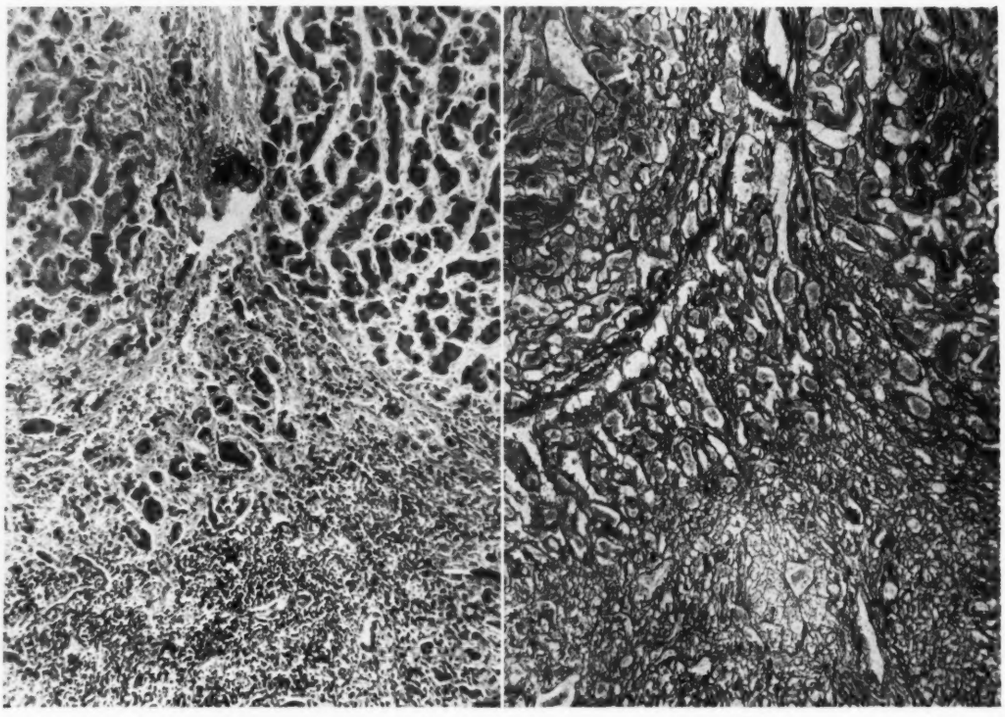

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FIG. 6. Case II. Subacute necrosis of the liver. Two nodules of regenerated parenchymal cells are seen in the upper corners of the picture with massive plasmacytic infiltration of the necrotic area in the lower portion; Giemsa stain, approximately 90 ×.
FIG. 7. Case II. Same area as Figure 5 stained by the Foot and Foot method for reticulum and showing the disorganization of the architecture, approximately 90 ×.

hyperglobulinemia, the globulins being 10.7, 11.2 and 9.5 gm. per 100 cc. in three determinations. No further studies were made of this protein. The thymol turbidity test was positive, the prothrombin time prolonged and there was moderate jaundice. The serum cholesterol was 60 mg. per cent with esters representing 41.5 mg. per cent. Blood cultures were negative until those taken on the last two days of life grew E. coli communis in all media inoculated. The electrocardiogram was normal except for slight left axis deviation and tachycardia. The phenomenon of sickling was readily demonstrated in this patient. Examination of blood from his mother, grandmother and sister, however, failed to reveal sickling.

Extensive x-ray studies failed to clarify the problem. Films of the skull and barium studies of the entire gastrointestinal tract were unremarkable. There was a small shadow of pleural thickening in the right costophrenic angle. Films of the long bones, entire spine and hands revealed trabecular markings which were coarse and prominent. In addition, two osseous defects were noted. One was irregular in shape and involved the lateral aspect of the distal end of the right tibia. The other was a small punched-out area on the medial surface of the distal end of the left femur. In neither area was there soft tissue reaction. The lesions were regarded as bone cysts by the radiologist and no suspicion of myeloma or of malignancy was entertained. Further hematologic studies revealed that the anemia was hyperchromic and both macrocytic and microcytic. Ascites appeared.

While these investigations were under way the patient suddenly became much sicker and his temperature rose. It was believed that he was developing pneumonia so penicillin was administered. Treatment previous to this had consisted of vitamin K and repeated transfusions of whole blood. He died with bleeding from the nose and mouth on June 28th, the morning after the first day of fever and roughly six months after the onset of symptoms.

Postmortem examination (autopsy no. 12464 performed twelve hours after death) revealed yellowness of the sclerae and pale striae of the skin over the shoulders, chest, abdomen, back and thighs, although the body seemed well

nourished with no other signs of wasting. There was a long, large keloid in the skin of the left abdomen at the operative site. A large, right, indirect inguinal hernia was filled with fluid which was easily expressed back into the abdomen. About a liter of chylous fluid was found in the abdominal cavity with no evidence of peritonitis. The spleen was absent and the site of the hilar resection was well healed. Each pleural cavity contained 150 cc. of clear yellow fluid. Twenty-three gm. of thymic tissue and a few slightly enlarged lymph nodes were found about the great vessels of the upper mediastinum. The heart weighed 220 gm. and appeared perfectly normal in all respects with no evidence of healed rheumatic or bacterial lesions. The lungs were heavy, weighing 1,450 gm., and there were scattered subpleural hemorrhages in all lobes. Edema fluid leaked from the bronchi and cut surfaces and scattered areas of the latter had a firm, glossy appearance, especially in the lower lobes. The hilar and bronchial lymph nodes were enlarged and red. The liver was also enlarged, weighing 2,000 gm., and was yellow with tiny, round, more intensely yellow, well circumscribed nodules scattered beneath the capsule. Raising the capsule on the inferior surface were two similar nodules, the larger being 1 cm. in diameter. Cross-section of the firm parenchyma revealed many nodules which were from 0.5 to 3.0 mm. in diameter and were scattered in a haphazard fashion. The parenchyma around the larger vessels were depressed. The intra- and extrahepatic biliary system was normal. There were obvious dilated thin-walled veins beneath the esophageal mucosa. The mesentery was edematous as were the prominent, markedly enlarged, reddish lymph nodes here and about the pancreas. In fact, all the lymph nodes examined were moderately to greatly enlarged, fairly soft and mottled red on section. The kidneys together weighed 390 gm. and were pale with slightly swollen cortices. There were bright red streaks in the pyramids. The pelves, ureters and bladder were normal. The marrow of the vertebral bodies, sternum, ribs and skull was pale red and of normal consistency. The osseous defects in the lower extremities were not examined. The brain appeared slightly edematous, weighing 1,400 gm., but was otherwise normal on gross and microscopic examination. Culture of the heart's blood taken postmortem grew E. coli.

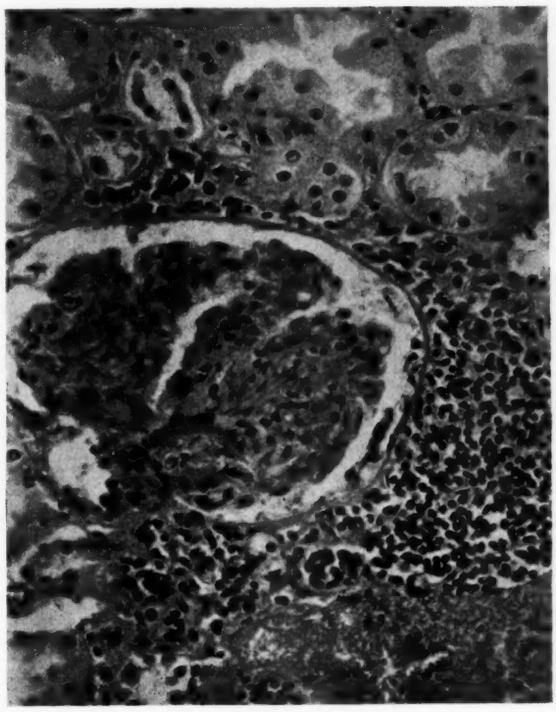
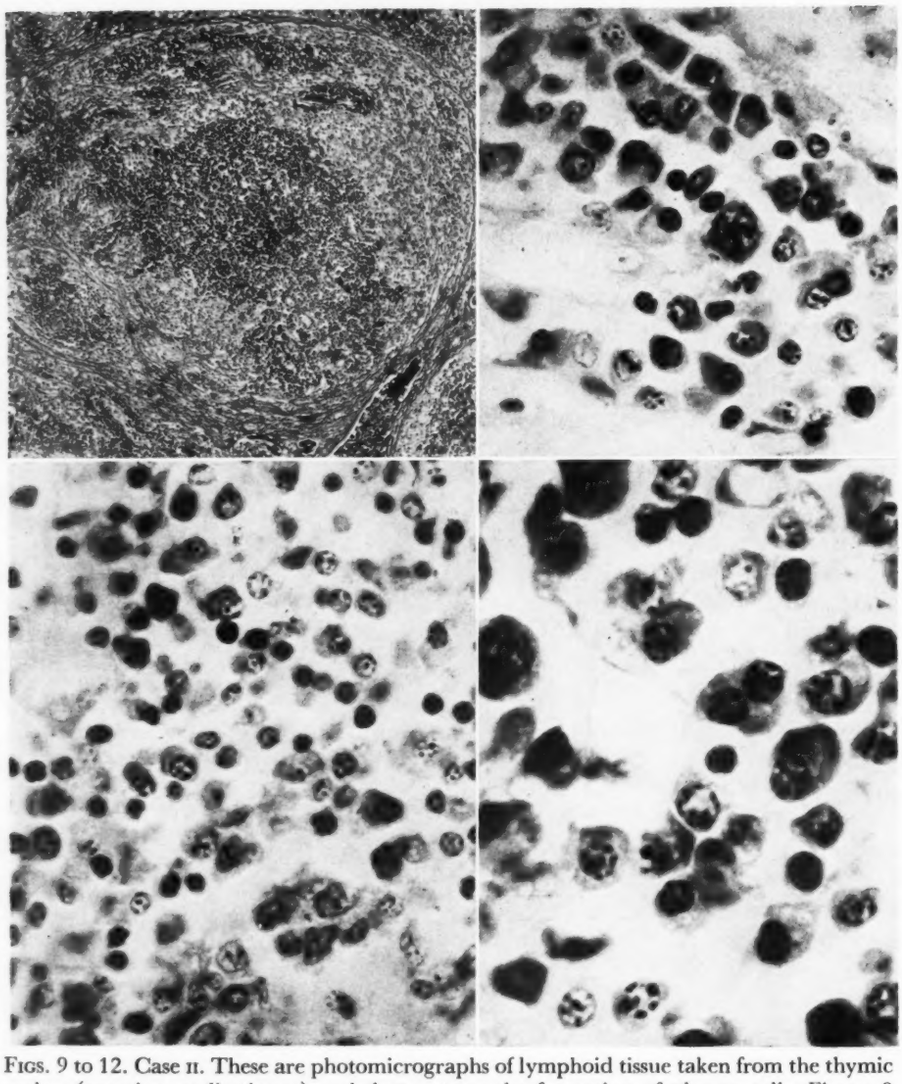


FIG. 8. Case II. Plasmacytic infiltration about a glomerulus, arteriole and thin-walled vein in the kidney. There is a small amount of precipitated protein in Bowman's space. Some of the endothelial cells of the glomerular membrane are swollen; hematoxylin and eosin stain, approximately 280 ×.

The microscope disclosed subacute massive necrosis of the liver, interstitial nephritis and plasmacytosis. The architecture of the liver was greatly distorted by areas of necrosis and collapse of the reticulum framework as well as by areas of regenerating liver tissue, some only the size of a high power field. (Figs. 6 and 7.) The most striking change, however, was the massive leukocytic infiltration which was composed almost exclusively of plasma cells, although scattered polymorphonuclear leukocytes were found around necrotic liver cord cells. This plasmacytic infiltration did not extend into the regenerating nodules and was most common as a "cuffing" in the portal zones of original lobules. The infiltration was largely interstitial and not intrasinusoidal; it did extend down into the lobules and around the central veins but to a lesser extent. Accompanying this there was a dropping out of many liver cord cells and degeneration and coagulation necrosis of others with a collapse of lobules and a consequent loss of architecture. In fact, few normal liver cells were found. Reticulum stains best revealed the lobular collapse and trichrome



FIGS. 9 to 12. Case II. These are photomicrographs of lymphoid tissue taken from the thymic region (anterior mediastinum) and demonstrate the formation of plasma cells. Figure 9 (approximately 50 ×) shows a lymphoid nodule with a center containing primitive cells and more mature cells appearing peripherally. The edema of the tissue is striking. The cellular details are shown in Figures 10 to 12; all erythrosin-azure-methylene blue stain. Figure 10 shows many mature and a few immature plasma cells in a peripheral sinus. The large cell is apparently a macrophage; approximately 810 ×. Figure 11, taken from near the center of the follicle, shows primitive cells in the lower right corner with increasing maturity of the plasma cells to the left and above these. Lymphocytes are also present; approximately 810 ×. Figure 12, taken from the periphery of the follicle, depicts immature and mature plasma cells with one mature binucleate cell. The vacuolization of the cytoplasm and the characteristic clumping of chromatin in the nucleus of the more mature cells are clearly visible; approximately 1,380 ×.

stains showed that a moderate amount of collagenous tissue had already been deposited. Canaliculae with inspissated bile were infrequent. The kidney changes were not spectacular although there was an extensive leukocytic infiltration, the chief cell of which was the mature plasma cell. Here again the infiltration was perivascular and periglomerular for the most part (Fig. 8), but the glomeruli and vessel walls were normal except for slight thickening and eosinophilic granularity. Some tubules were crowded by infiltrate and the convoluted tubular cells were slightly swollen. Many collecting tubules in the medulla contained desquamated cells and here the capillaries were congested. No true casts were seen.

Lymphoid tissue was in a sense transformed into an organ producing plasmacytes. Although

the lymph nodes were enlarged they did not have the usual hyperplastic appearance. Instead, the sinuses were distended and contained many red blood cells. In general, relatively few lymphocytes were present and the cortex and medullary cords were largely composed of plasma cells which also crowded the sinuses. Most of these were typical mature plasma cells; occasionally a binucleate cell was seen. (Fig. 12.) Focal plasma cell formation was indicated and several stages of development could be recognized. Apparent progression was traced from large, primitive "reticular" cells to large cells with slightly basophilic cytoplasm and more or less centrally located large nucleus with slightly clumped chromatin and with nucleoli. Next slightly smaller cells with eccentric nuclei, more clumped chromatin, nucleoli and similar cytoplasm were seen. Finally, there were typical adult cells which in this case predominated. Large and small lymphocytes were also present but no mitotic figures were seen. In a section of lymphoid tissue taken from the thymic region but not showing Hassall's corpuscles this production was evident and had almost completely replaced the lymphocytes. (Figs. 9 to 12.) With an erythrosin-azure-methylene blue stain, the extreme basophilia of the plasma cell cytoplasm was most clearly demonstrated. The vessels of the capsule of the adrenals and lymph nodes were cuffed with plasma cells. (Fig. 13.) Sections of marrow from vertebrae, sternum and skull showed moderately active hyperplasia of the normal elements, especially of the erythroid series and scattered immature and mature plasma cells, some of the latter being binucleate. Perivascular plasmacytic infiltration was seen also in other organs.

Sections of the spleen revealed a few mature plasma cells indiscriminately scattered through the section. The red pulp was largely obscured by laked red cells and hemosiderin. The follicles contained large, pale germinal centers and their cytology was unremarkable.

Throughout all tissues there was intravascular precipitation of protein which was unusual in the large size of the granules formed. This was most striking in the lungs where the protein of the edema fluid precipitated either as large granules or, in other areas, agglomerated with intra-alveolar hemorrhages to form solid sheets which evidently accounted for the glassy appearance seen in the gross. Sections of the heart were entirely normal. Many of the red cells seen within vessels in the various sections assumed a form similar to sickled cells.

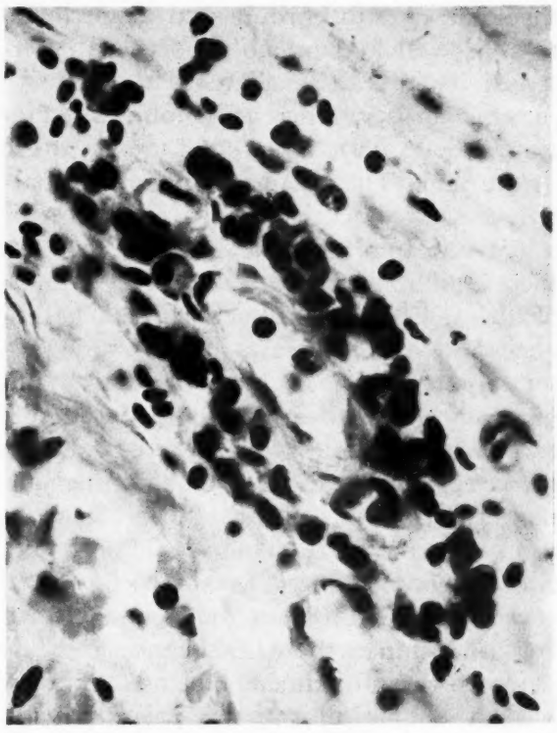


FIG. 13. Case II. Perivascular cuffing in the capsule of a lymph node by plasma cells, similar to Figure 4 in Case I. The plasma cells here are, in the main, mature. Compare with the small lymphocyte and young polymorphonuclear leukocyte in the lumen of the vessel; hematoxylin and eosin stain, approximately 700 ×.

The major final diagnoses were: hypersensitivity reaction (possibly due to sulfadiazine); subacute massive necrosis of the liver with ascites, edema of the lungs and jaundice; plasmacytosis with hyperglobulinemia; interstitial nephritis; anemia; thrombocytopenia; sicklemia.

COMMENTS

The anatomic demonstration of diffuse plasmacytic infiltration associated with hepatitis, interstitial nephritis and interstitial myocarditis in the first case in which the history was strongly suggestive of hypersensitivity to sulfadiazine led to a re-evaluation of the second case in which the anatomic findings were so similar. The latter had been at first regarded as an atypical multiple myeloma and it required a careful review of the clinical chart and the report from the first hospital to elicit the history as presented. Although the relationship of increased globulin formation to multiple

myeloma and to chronic and acute infections is well known, no reports of hypersensitivity in man associated with extensive plasmacytosis and increased globulin formation were found in a search of the literature at the time. Then Teilum's papers on paramyloidosis, Boeck's sarcoid and disseminated lupus erythematosus appeared.[6,7] In these Teilum enlarged upon his hypothessi of allergic hyperglobulinosis in the pathogenesis of these diseases and briefly presented several cases. One of these was that of a nine year old child who died of Letterer-Siwe's disease six and one-half years after a severe reaction to vaccination against smallpox, and the other was that of a fifty-eight year old man who died in uremia more than twenty months after sulfonamides had been administered in the treatment of pneumonia. In both instances there had been an increase in serum globulins during life and at autopsy plasma cellular infiltrations were prominent. In the latter case the lesion at autopsy was paramyloidosis of the kidney, spleen, liver and myocardium and was regarded by Teilum as probably secondary to hypersensitivity to the sulfonamide. Later during the preparation of this report Carter's case of plasmacytosis and hyperglobulinemia in trichinosis was published.[3]

Although the lesions in the cases under discussion are attributed to hypersensitivity to sulfadiazine, it must be noted that both patients also received penicillin over long periods and that a reaction to penicillin cannot be rigidly excluded. Although cutaneous reactions to penicillin are well known, a search of the literature has revealed no autopsied cases of hypersensitivity with similar lesions following its use.

In the first case the probability of hypersensitivity was realized by the clinicians late in the course although its importance escaped them. In the second case, we must, if we are to indict a sulfonamide as the etiologic agent, either suppose that there had been exposure to a sulfonamide drug at some time before the fatal illness, that the ointment used in healing the initial wound was a sulfonamide ointment, or that intermittent administration of the drug for a period of only a few days brought on the florid response. It is also necessary to presume that the allergic response, once initiated, progressed without further stimulation by added antigen. This phenomenon, however, is not unknown in the field of allergy as Burnet shows in his monograph[8] using the measles experience of the Faroë Islanders and yellow fever as examples. When we consider that poultry often receive sulfonamides in their feed as a prophylactic measure and that the sulfonamides are indiscriminately employed by physicians and laity alike, it is evident that most people are exposed to these drugs in one way or another. Sulfonamide ointments are extremely prone to produce hypersensitive reactions, as Sulzberger, Kanof, Baer and Lowenberg[9] have demonstrated.

Pathology and Pathogenesis. Hageman and Blake[10] and Goodman and Levy[11] were among the first to suggest hypersensitivity as a factor in the reactions of individuals to sulfonamides. Rich and Gregory[12] demonstrated the similarity between the lesions of serum sickness in rabbits and serum and sulfonamide hypersensitivity reaction in man. Experimental studies have revealed that sulfonamides bound to proteins have antigenic properties.[13] The various reports of human and animal sensitivity to sulfonamides[13–19] have described all of the lesions found in these cases except that of plasmacytosis. Rash, fever, anemia, leukopenia and hemorrhages are classical findings. Hepatitis, interstitial myocarditis and interstitial nephritis are common but the previous descriptions of the cellular exudate in these organs fail to reveal the plasma cell as the predominant cell type. Most of the reported cases are of illnesses of a short duration, as short as five days in several instances, and so would be expected to show more acute lesions, so-called anaphylactic responses. In the first case presented here the patient lived for nineteen days after drug fever appeared; in the second case the illness persisted for six months. In many infectious processes plasma cells do not appear in the

exudates until the lesion has existed for a period of days or until healing is apparent. Several authors have described a slight plasmacytic response in cases of sulfonamide hypersensitivity in which the symptoms existed for several weeks. French[16] described as the predominant cell in his series an "acidophilic histiocyte," many of which were to him distinguishable from plasma cells only by their acidophilic cytoplasm. In the cases under discussion the older plasma cells had a rather eosinophilic, homogeneous cytoplasm with the hematoxylin and eosin stain but had typical basophilic cytoplasm with Giemsa or erythrosin-azure-methylene blue stains. It is notable that the plasmacytic accumulations in the more acute case were composed of fewer mature cells than in the more chronic case in which most of the plasma cells were mature. Perhaps a plasmacytic response is an indication of relative chronicity of the lesion in hypersensitivity as well as in infectious processes. In the second case an eosinophilic response was prominent early in differential peripheral blood counts.

The hepatitis in the more chronic case was subacute with many foci of regenerating liver cells, and the patient died of liver failure. Although this has not previously been reported, acute hepatic necrosis such as that seen in the first case has been observed. The necrosis of liver cells may represent a direct antigen-antibody response or may be secondary to vascular damage but as yet there is no experimental evidence to support either theory. The myocarditis of the first case is typical except for the type of cellular infiltration. Similar myocarditis may have been present in the second case early in the course when electrocardiographic changes were evident and may then have healed by resolution leaving no scarring of the myocardium, since it is generally held that the myocarditis of sulfonamide hypersensitivity is reversible. The absence of changes in the myocardium and valves at the time of autopsy would seem to indicate that there had been no rheumatic fever or bacterial endocarditis at the time when the blood cultures grew streptococci. The interstitial nephritis in both cases is also characteristic except for the cellular response. In the first case oliguria and uremia led to the fatal termination. Trabeculitis in the spleen was first described by More, McMillan and Duff[18] and was evident in this case. It probably occurs as a consequence of a vascular lesion. Examination of available sections of the surgically removed spleen in the second case failed to reveal any noteworthy lesions. Perivascular cuffing by leukocytes has been described in sulfonamide hypersensitivity by most investigators and often accompanies vascular necrosis. Generalized vasculitis may be an acute response on the part of the patient as it is commonly seen in cases of very short duration. No vascular necrosis is evident in these cases but such lesions may have existed earlier. The perivascular accumulation of plasmacytic cells is prominent. The "granulomatous" lesions described by More et al.[18] are not apparent.

Hyperglobulinemia, Plasmacytosis and Hypersensitivity. Hyperglobulinemia was a prominent feature of the second case and in the first case the serum globulins were increasing at the time of death. The association of plasma cell myeloma and Bence-Jones proteinuria was known and investigated for many years. With the ability to study serum proteins in the clinic the fact became obtrusive that increased or abnormal serum globulins are associated with a large number of conditions in which plasma cells accumulate. The Scandinavian workers, beginning with Bing,[1] have made the majority of experimental and clinical investigations of the relationship between hypersensitivity, hyperglobulinemia and plasmacytosis. The most extensive of these is that of Fagraeus[4] which presents a comprehensive survey of the literature with many of her own detailed *in vivo* and *in vitro* studies. Bjorneboe, Gormsen and Lundquist[2] found an intense plasmacytic infiltration (without accompanying myocarditis, hepatitis or nephritis) in a number of internal organs of rabbits after long-continued hyperimmunization

with pneumococcus vaccines. From the experimental evidence now available it may be assumed that proliferation of cells of the plasmacytic type is associated with the production of antibody globulins, and probably with abnormal globulins. That these are the only cells so concerned has not been proved. Whether the antibody globulins are produced by immature or mature plasmacytic cells or by both is still conjectural.

In the first case presented here the blood sulfonamide level on the day before death was 17.4 mg./100 cc. so that in this case also ample antigen was present even at this time to stimulate plasmacytosis and hyperglobulinemia as well as nephritis, hepatis and myocarditis. In the second case no exogenous antigen in the form of sulfonamides had been received by the patient for almost five months before death. The evidence that sensitivity to sulfadiazine was responsible for the plasmlcytosis, hyperglobulinemia and organic damage is suggestive but incomplete.

Fever, anemia, leukopenia and eosinophilia were present immediately after the second treatment with sulfadiazine; liver damage and myocarditis become evident within a month. The plasmocyte response and the level of serum globulin at this time are unknown. Marked hyperglobulinemia was evident when first investigated three and one-half months after treatment and persisted throughout the remainder of the illness. Massive plasma cell infiltration was evident at autopsy. If the sulfadiazine was responsible, it must be assumed that the plasma cell reaction persisted for months after the withdrawal of the drug. Immunologically it is necessary to assume that antibody formation continued after the original offending antigen had disappeared. The possibility that this sequence may occur with other antigens has been suggested by Burnet.[8] Possibly the chief reason for considering this a reaction to an antigen, in this case to sulfadiazine, rests on the large amount of experimental work in animals which shows that plasmacytosis and hyperglobulinemia are responses to a great variety of antigenic stimuli.

Origin of the Cells. In these cases the plasmacytic cells apparently developed from primitive cells in the lymph nodes, bone marrow and spleen, and possibly in other sites, but no generalizations concerning the origin of plasma cells are permissible from this study.

Differential Diagnosis. The differentiation between plasma cell myeloma and massive plasmacytosis is difficult, as was shown in Carter's case of trichinosis and plasmacytosis, and in Case II. In many cases the distinction will be readily determined. The difficulties in interpretation of borderline cases, however, are present during life and even after postmortem examination. A sternal aspiration or a tissue biopsy exhibiting proliferation of plasma cells is often considered diagnostic of myeloma regardless of the morphology of the cells present. When essentially normal cells of the plasmacytic series are found in moderate numbers along with normal marrow elements in a case exhibiting anemia and hyperglobulinemia, it is wise to consider a diagnosis of plasmacytosis before applying the ominous label of multiple myeloma. Whether the plasmacytosis of hypersensitivity may lead to plasma cell myeloma remains to be proved.

It is only by careful evaluation of the history and of the histologic findings that a definite diagnosis may be made. Although the histologic differentiation between these two conditions cannot be fully discussed here, some of the points that may aid the investigator are these. In plasmacytosis there is a non-neoplastic distribution of the plasma cells in an infiltrative rather than an invasive manner. Tumor masses are not found in the bone marrow or viscera. Leukostasis in the blood vessels, especially in the lung, liver, spleen and kidneys, is not apparent. Abnormal (neoplastic) forms are not present. Finally, the whole spectrum of the development of the plasma cell, including typical mature cells, can be found in lymph nodes, spleen and bone marrow.

We have observed in this laboratory a considerable number of autopsied cases in which hypersensitivity, hyperglobulinemia

and plasmacytosis have played a part in the patient's illness. There have, however, been no other autopsies presenting the combination of plasmacytosis and the other extensive and fatal lesions present in the two cases described here in the 7,000 postmortem examinations made at the New York Hospital since 1932. In none of the eleven cases of multiple myeloma autopsied during this period has there been a history suggestive of hypersensitivity.

SUMMARY

The clinical and postmortem findings in two cases of probable hypersensitivity to sulfadiazine have been presented. One patient survived for more than five months after the onset of symptoms whereas the other survived only nineteen days. The lesions in both cases consisted in the main of widespread plasmacytosis, hepatitis and interstitial nephritis. The case with the longer survival had marked hyperglobulinemia during life. This patient died of liver failure and the other of kidney failure. In neither case was the diagnosis of hypersensitivity fully appreciated during life. The relationships of hypersensitivity, hyperglobulinemia and plasmacytosis are discussed, along with the difficulties in differential diagnosis of the disease processes in which they occur. These are the first cases to be presented in which sulfonamide drugs have been indicted as probably responsible for extensive plasmacytosis and hyperglobulinemia in addition to the lesions commonly seen in sulfonamide hypersensitivity.

Acknowledgment: The autopsy in Case 1 was performed by Dr. Marjorie Allen, who determined the essential nature of the disease processes. The photographs were prepared by Mr. Julius Mesiar. The sections of the spleen in Case II were made available by Dr. Arthur R. Abel.

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Relationship of Bone Marrow Plasmacytosis to the Changes in Serum Gamma Globulin in Rheumatic Fever*

ROBERT A. GOOD, M.D.† *and* BERRY CAMPBELL, PH.D.

Minneapolis, Minnesota *New York, New York*

ALTHOUGH Swift and his co-workers,[1] among others, long ago pointed out that leukocytosis of the peripheral blood could be used as an index of disease activity in rheumatic fever, little work has been done to define in detail the condition of the hematopoetic tissues in this disease. Neither the commonly occurring anemia[2] nor the consistent leukocytosis[3] observed in rheumatic fever has provoked quantitative studies of the bone marrow in children with this disease. Eosinophilia of the peripheral blood has been reported to occur frequently in patients with chorea[4] and erythema multiforme but has not been reported in patients with active rheumatic fever. Mild to moderate plasmacytosis of the peripheral blood occurring one to three weeks after the onset of scarlet fever has been described[5] and it has been shown to be more frequent and quantitatively greater in patients developing "complications" following streptococcal disease than in those escaping sequelae.[6] Impressive evidence[7–13] has accumulated indicating that the group A streptococci or their products are somehow involved in the chain of events leading to clinically recognizable rheumatic fever. Studies of the specific antibodies against streptococci and the soluble antigens produced by them,[14–18] as well as studies of the serum gamma globulin,[17] in patients developing rheumatic fever have provided corroborating evidence of the association of streptococcal disease and rheumatic fever. In addition these studies have indicated that the elaboration of antibodies and the release into the blood of significant increments of gamma globulin are often, if not regularly, taking place during the acute exudative phase of this disease. Not infrequently the production of gamma globulin is pronounced.

In an attempt to define the characteristics of the bone marrow in rheumatic fever, streptococcal pharyngitis and chorea, as well as to gain evidence on the relationship of the bone marrow alterations to elaboration of antibodies and release of gamma globulin in these diseases, the following studies were carried out.

MATERIALS AND METHODS

Aspiration biopsies of the sternal bone marrow were performed on twelve normal children, twenty-two children with acute rheumatic fever, fifteen convalescent from rheumatic fever, six with inactive rheumatic fever, eight with acute Sydenham's chorea, six convalescent from chorea, six with acute streptococcal pharyngitis and three convalescent from this disease. Serum gamma globulin levels estimated by Kunkel's[19] $ZnSO_4$ turbidimetric method were obtained at least once on all these children and at weekly intervals during the acute phase of the rheumatic fever and chorea. The use of this relatively simple method of estimating gamma globulin levels is justified by the good agreement of the results with those obtained by electrophoresis, as shown by Kunkel and confirmed in our laboratory.

* From the Department of Pediatrics and Anatomy, University of Minnesota, Minneapolis, Minn., and the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.

† Helen Hay Whitney Foundation Fellow in Rheumatic Fever Research.

The "normal" children studied comprised a group of patients admitted to the Pediatric Service at the University of Minnesota Hospitals for surgical correction of minor congenital anomalies (squint, cleft palate, indirect inguinal hernia), for study of psychogenic behavior problems or for investigation of acyanotic congenital heart disease. All of these children were otherwise entirely well at the time of study. None had suffered recently or were suffering from respiratory infection which could be discovered by careful medical history, thorough physical examination or routine laboratory studies of the blood and urine. The patients with acyanotic heart disease chosen for study were those who had shown no evidence of growth disturbance nor any physical deficiency in consequence of their anomalies.

The patients included in the category of inactive rheumatic fever were children with a cleancut history of past rheumatic disease who showed evidence of organic heart damage as a residuum of the previous illness but were perfectly well at the time of study and had been free of recognizable infection during the two months prior to the time of sampling.

The major rheumatic manifestations recorded in the patients with active rheumatic fever included migratory polyarthritis, subcutaneous nodule formation, characteristic carditis and chorea; while the minor manifestations were fever, elevated erythrocyte sedimentation rate, leukocytosis, epistaxis, erythema marginatum, erythema nodosum and erythema multiform. In most of the patients the erythrocyte sedimentation rate exceeded 90 mm. in one hour (Westergren) and in only one patient, a five year old child in heart failure, was the sedimentation rate below 50 mm. in one hour. No patients were included unless at least one major manifestation and two minor manifestations were obviously present. Most of the patients studied had at least two major and three minor manifestations. Serial bone marrow biopsies as well as serial determinations of serum gamma globulin were obtained on as many of these rheumatic children as was possible. Of the twenty-two original patients suffering from acute rheumatic fever, we were able to follow fifteen throughout their disease to rheumatic quiescence.

The patients included in the group designated "streptococcal pharyngitis" were children suffering from acute sore throat with fever, moderately elevated erythrocyte sedimentation rate and leukocytosis, and from whose throats large numbers of β-hemolytic streptococci were cultured. Two of these six patients had scarlet fever. In only three of the six was it possible to obtain a second bone marrow biopsy two or three weeks after subsidence of the pharyngeal angina.

No medications were given to any of the children before the initial marrow biopsy was obtained or the initial blood sample drawn. The patients suffering with acute rheumatic fever were treated with moderate, symptom-relieving dosages of salicylates during the period of clinical activity of the rheumatic episode. The patients who had chorea were treated with bed rest and moderate dosages of barbiturates as needed. The children in the convalescent and inactive rheumatic fever groups received no medication during the course of the study. Following the initial bleedings and bone marrow biopsies the patients with streptococcal pharyngitis were treated either with intramuscular injections of aqueous penicillin G or with orally administered sulfadiazine in therapeutic dosages until the symptoms of infection had abated.

All bone marrow biopsies were taken from the body of the sternum with a Klima-Rosegger[20] type needle. This needle has the advantage over other needles used for sternal biopsy of having an adjustable metal guard which serves at once to provide a grip for insertion of the needle and to protect the patient against accidental penetration of the inner lamella of the sternal bone. Such a needle is virtually essential for satisfactory studies of bone marrow in children. Upon gaining entrance to the marrow cavity a 5 cc. syringe was attached to the needle and a negative pressure, produced by withdrawing the plunger of the syringe to the 3 cc. mark, was applied while 0.1 cc. of marrow was aspirated. A tiny sample of the marrow so obtained was smeared on a clean glass slide to prepare a smear of tissue like that shown in Figure 1. The advantages of this preparation for quantitating the less abundant marrow elements are obvious. Using a small sample of marrow with none of the marrow overlapping the edges of the glass slide, a satisfactory sampling of the tissue can be accomplished by the use of counts which are technically feasible. The marrow was fixed by whipping the slides rapidly in the air and staining was done with the Wright-Giemsa combination of Downey.

Figures for the relative percentages of the

bone marrow elements were obtained from counts of 500 cells from each marrow. Because it is universally recognized that quantitative evaluation of the less abundant marrow elements is hazardous unless large numbers of cells are counted, the scheme employed for quantitation

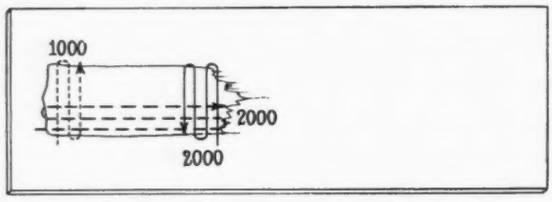

FIG. 1. Diagrammatic representation of the bone marrow smears on the microscopic slide as used for estimating the relative number of marrow cells and the number of plasma cells. Arrows indicate part of smear in which counting is done and the figures indicate the numbers of nucleated cells counted in each area.

of the plasmacytes was to record the total number of plasmacytes per 5,000 nucleated marrow cells counted. Two thousand cells were counted across the feathered end of the smear, 2,000 along the longitudinal axis and 1,000 across the base of the preparation. (Fig. 1.) In this way the irregularities of cellular distribution due to smearing the tissue are well compensated and the plasmacyte counts obtained are reproducible and appear to be highly reliable. Differential studies of the plasmacytes encountered during the process of the 5,000 cell counts were also recorded. In Figure 2 are illustrated examples of the several types of plasmacytes observed in the bone marrow of the patients with rheumatic fever.

The terminology used in this paper represents a modification of the terminology used in our previous publications in order to attain agreement with that suggested by the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-forming Organs. The term plasmablast used here refers to the same cells we[21] previously called plasma cellular reticulum after Rohr. Proplasmacytes are the same cells we have previously called reticular plasma cells. The large, medium and small plasmacytes are those previously called large, medium and small plasma cells, respectively.

RESULTS

In Table I is recorded a summary of the relative percentages of the various cellular elements found in the bone marrows of these patients. The results obtained in this study are compared to the normal values given for adults by Wintrobe.[22] It would appear from these data that the bone marrow of normal children in the age group studied differs little from that of normal adults. It would further seem that the marrow of children with acute rheumatic fever is not strikingly different from that of normal children or normal adults in most respects. This finding is somewhat surprising in view of the well known alterations in the peripheral blood in this disease. The tendency shown for the myeloid-erythroid ratio to be slightly increased in patients with both rheumatic fever and acute streptococcal pharyngitis is, however, in keeping with the observed alterations in the peripheral blood in these diseases.

No significant variations in either the morphology or the relative percentages of eosinophiles was noted in any of these groups of patients. Careful examination of the original data revealed, however, that four patients, each with an erythematous skin rash, showed eosinophilia of the bone marrow. Because of additional co-existent symptomatology three of these patients, one with erythema nodosum and two with erythema marginatum, were included in the rheumatic fever group while one with erythema multiforme was included among the patients with streptococcal pharyngitis.

One consistent difference between the bone marrow of rheumatic patients and that of the normal children is, however clearly apparent from these data. In children with active rheumatic fever an increase in the number of bone marrow plasmacytes regularly occurs. A non-significant trend in the same direction appears in the streptococcal pharyngitis group. Normal numbers of plasmacytes, on the other hand, are characteristic of the bone marrow in patients with uncomplicated Sydenham's chorea and inactive rheumatic fever. A more exact and extensive study of the bone marrow plasmacytes is to be seen in Table II in which the mean numbers of plasmacytes per 5,000 nucleated marrow cells for

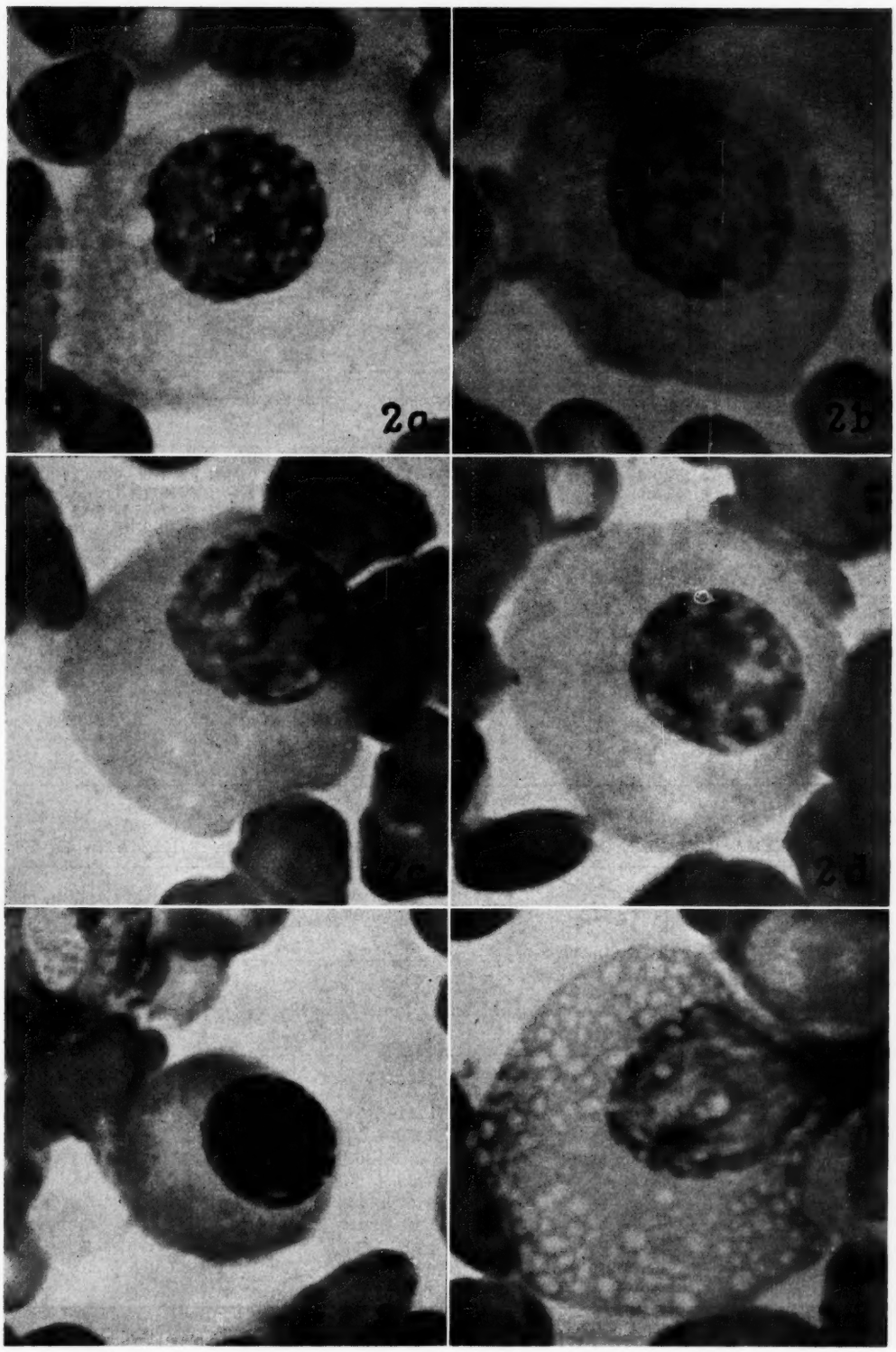

FIG. 2. Representative plasmacytes taken from the bone marrow of a patient with acute rheumatic fever. This figure illustrates the sequence of cellular development in the plasmacyte line; (*a*) is a plasmablast; (*b*) an early proplasmacyte; (*c*) a late proplasmacyte; (*d*) a large mature plasmacyte; (*e*) a medium-sized mature plasmacyte; (*f*) illustrates one of the plasma cell variants observed in the marrow of rheumatic children, a plasmacyte with Russell bodies.

each group of patients is recorded. It may readily be seen that an increase in bone marrow plasmacytes occurs in acute rheumatic fever and in patients convalescent from streptococcal pharyngitis. A consistent tenfold increase in the numbers of plasmacytes in the marrows of children with acute rheumatic fever is shown, while in those convalescent from rheumatic fever a return toward normal numbers of bone marrow plasmacytes is seen. Children with inactive rheumatic fever as well as those with active and inactive Sydenham's chorea show no deviation from the normal plasmacyte content of their marrows. A slight plasmacytosis of borderline significance occurs during streptococcal pharyngitis which is followed by what appears to be a more significant increase in plasmacytes during convalescence from this disease. Among the patients in the convalescent stage of rheumatic fever only one showed a significant plasmacytosis at the time of study. In Table II the studies of serum gamma

TABLE I

RELATIVE NUMBERS OF CELLS IN BONE MARROW

Cell Types	Normal Children 2.5–15 yr. (13 cases)		Acute Rheumatic Fever (15 cases)		Acute Chorea (7 cases)		Streptococcal Pharyngitis (6 cases)		Inactive Rheumatic Fever (6 cases)		Wintrobe's[22] Normal Adults	
	Mean %	Range %	Mean %	Range %	Mean %	Range %	Mean %	Range %	Mean %	Range %	Mean %	Range %
Myeloblasts	1.25	0.7– 2.2	0.97	0.6– 1.5	0.91	0.8– 1.0	1.30	0.6– 1.8	1.00	0.4– 1.4	2.0	0.3– 5.0
Promyelocytes	4.50	3.0– 6.0	3.59	2.2– 5.8	3.28	2.0– 4.0	4.30	3.6– 5.6	3.73	1.8– 4.8	5.0	1.0– 8.0
Neutrophile myelocytes	16.33	8.4–23.4	13.50	8.0–17.6	12.80	10.2–14.0	16.83	9.0–21.2	13.95	10.6–19.0	12.0	5.0–19.0
Eosinophile myelocytes	2.02	0.8– 3.6	2.70	0.4– 7.4	1.60	0.4– 2.4	2.03	0.8– 3.6	0.97	0.4– 1.2	1.5	0.5– 3.0
Basophile myelocytes	0.48	0.0– 1.4	0.22	0.0– 0.8	0.29	0.0– 0.6	0.43	0.2– 0.8	0.27	0.0– 0.4	0.3	0.0– 0.5
Neutrophile metamyelocytes	30.10	21.8–35.8	26.06	19.0–33.4	25.9	21.4–31.2	33.03	28.4–38.2	26.50	24.2–30.0	22.0	13.0–32.0
Eosinophile metamyelocytes	1.51	0.6– 2.6	1.90	1.2– 3.8	1.30	0.4– 2.2	1.93	0.2– 6.0	1.33	0.6– 3.0	....	.....
Basophile metamyelocytes	0.32	0.0– 1.0	0.13	0.0– 0.4	0.25	0.2– 0.4	0.30	0.0– 0.6	0.63	0.0– 1.0	....	.....
Mature neutrophiles	10.20	3.7–14.6	16.68	9.8–25.8	13.34	7.0–17.8	14.16	4.2–24.2	15.63	11.6–21.6	20.0	7.0–30.0
Mature eosinophiles	1.15	0.4– 2.2	1.45	0.2– 3.5	1.39	0.6– 2.6	1.30	0.2– 3.6	0.83	0.0– 2.0	2.0	0.5– 4.0
Mature basophiles	0.28	0.0– 0.6	0.27	0.2– 0.4	0.32	0.2– 0.6	0.28	0.0– 0.8	0.33	0.2– 0.8	0.2	0.0– 0.7
Lymphocytes	10.08	4.4–17.0	11.13	5.6–21.0	14.20	11.4–19.0	8.10	4.6–13.8	11.50	8.4–16.6	10.0	3.0–17.0
Plasma cells	0.41	0.0– 1.0	4.04	2.5– 7.2	0.31	0.2– 0.8	0.90	0.6– 1.2	0.53	0.2– 1.4	0.4	0.0– 2.0
Reticulum cells and monocytes	1.66	0.4– 3.0	1.27	0.2– 2.2	1.46	0.6– 2.0	1.40	0.4– 2.4	1.03	0.4– 1.4	2.2	0.7– 7.0
Megakaryocytes	0.43	0.2– 1.0	0.36	0.0– 0.6	0.43	0.2– 0.6	0.27	0.2– 0.4	0.40	0.0– 1.2	0.4	0.3– 3.0
Pronormoblasts	1.96	0.6– 3.0	1.26	0.4– 1.8	1.43	0.8– 2.0	1.63	0.6– 2.6	1.90	1.0– 3.0	4.0	1.0– 8.0
Normoblasts	17.27	11.0–21.0	15.23	6.4–26.0	20.54	15.8–22.8	12.65	9.2–18.0	19.03	16.4–25.4	18.0	7.0–32.0

TABLE II

SERUM GAMMA GLOBULIN AND BONE MARROW PLASMA CELLS IN RHEUMATIC FEVER

Clinical Description	Age in Years	No. of Cases	Gamma Globulin gm. %				Total Plasma Cells per 5,000 Nucleated Marrow Cells			
			Mean	Range	S. D.	S.E. of Mean	Mean	Range	S. D.	S.E. of Mean
Normal children	2½–15	12	0.78	0.62–1.05	.13	.04	19.8	11–32	8.7	2.5
Acute rheumatic fever	5–13	22	1.85	1.11–2.54	.47	.10	170.0	98–320	54	11.5
Convalescent rheumatic fever	5–13	15	0.86	0.70–1.11	.11	.03	39.1	18–75	14.8	3.8
Active Sydenham's chorea	6–11	8	0.86	0.70–1.02	.11	.04	23.0	15–33	6.4	2.8
Convalescent Sydenham's chorea	6–11	7	0.78	0.59–1.00	.13	.05	24.8	17–31	4.7	1.8
Acute streptococcal pharyngitis	3–15	6	0.75	0.69–0.92	...	...	35.1	24–53	....	....
Convalescent streptococcal pharyngitis	5–7	3	1.13	1.00–1.31	...	...	60.0	45–72	....	....
Inactive rheumatic fever	7–19	6	0.76	0.71–0.86	...	...	27.8	11–45	....	....

globulin levels are also summarized. It is apparent that the serum gamma globulins, like the bone marrow plasmacytes, show an elevation only in the patients with active rheumatic fever and in those convalescent from streptococcal pharyngitis. The consistent bone marrow plasmacytosis in rheumatic fever is strikingly correlated with the consistent elevation of gamma globulin in the sera of these patients. In Table III the correlation of the magnitude of the gamma globulin alterations with the magnitude of the plasmacytosis is illustrated. In arranging the patients suffering from acute rheumatic fever according to increasing serum gamma globulin levels, the figures representing total plasmacytes in the bone marrow of these patients also appear in roughly ascending order. These data indicate that in patients with rheumatic fever there is a correlation both between plasmacytosis of the bone marrow and elevation of serum gamma globulin, and between the degree of hypergammaglobulinemia and the degree of plasmacytosis.

In Table IV a differential study of the type of plasmacytes present in the bone marrow of these patients is reported. It is clear from this table that in patients with active rheumatic fever, who have been shown to be forming increased amounts of gamma globulin, there is an increase in both the young and the older plasmacyte types. The plasmacyte types present in the marrow have approximately the same percentage relationship to each other as occurs in the bone marrow of normal children. In the group of patients convalescent from acute rheumatic fever there seems to be a relative preponderance of young cell forms, while a return toward the normal relationship is seen in the patients with inactive rheumatic fever. A relatively high percentage of lymphoid plasmacytes seems to occur in patients with streptococcal pharyngitis while fewer mature plasmacytes are

TABLE III

CORRELATION OF PLASMA CELL COUNT AND LEVEL OF SERUM GAMMA GLOBULIN IN RHEUMATIC FEVER

Patient	Age	Sex	Gamma Globulin gm. per cent	Plasma Cells per 5,000 N.C.	E.S.R.	Major Rheumatic Manifestations
1. H. O.	10	M	1.11	121	78	Polyarthritis, carditis
2. L. W.	9	M	1.17	116	52	Arthritis, pharyngitis
3. T. M.	25	F	1.26	92	92	Polyarthritis, carditis
4. C. S.	10	F	1.36	121	95	Chorea, carditis
5. T. C.	6	M	1.38	98	106	Polyarthritis
6. G. V.	10	F	1.44	128	82	Polyarthritis, carditis
7. V. Z.	5	F	1.44	205	102	Polyarthritis, carditis
8. M. S.	5	F	1.52	120	20	Carditis, subcutaneous nodules, heart failure
9. S. G.	5	F	1.61	120	126	Carditis, polyarthritis
10. G. A.	11	M	1.61	193	96	Carditis, polyarthritis, E. marginatum
11. J. D.	13	M	1.64	137	101	Polyarthritis, carditis
12. B. K.	13	F	1.72	183	100	Polyarthritis, carditis
13. K. A.	10	F	1.72	214	86	Carditis, subcutaneous nodules
14. S. S.	5	M	1.88	166	104	Polyarthritis, subcutaneous nodules, carditis
15. C. C.	12	M	1.99	174	112	Polyarthritis, carditis
16. R. E.	16	M	1.99	214	106	Polyarthritis, carditis
17. R. W.	10	F	2.26	320	98	Chorea, carditis, polyarthritis
18. E. A.	12	M	2.32	122	74	Polyarthritis, subcutaneous nodules, E. marginatum
19. D. M.	12	M	2.32	144	122	Polyarthritis
20. P. E.	9	M	2.32	162	98	Polyarthritis, carditis, E. nodosum
21. L. N.	14	M	2.54	175	120	Carditis, polyarthritis
22. S. R.	10	F	2.64	258	96	Polyarthritis, carditis
23. R. H.	16	M	2.70	249	126	Polyarthritis, carditis

seen in the marrows of these patients than in any of the other groups.

In Figure 3 the clinical course of a typical patient of this series is depicted. This thirteen year old female suffered a severe acute sore throat two to two and a half weeks prior to admission to the University of Minnesota Hospitals. Three days prior to admission she developed hot, red, swollen, painful, tender ankles. The polyarthritis had migrated to involve the knees, wrists and left elbow at the time of admission. This figure compares the temperature course, curves for erythrocyte sedimentation rate and serum gamma globulin to the graph representing the plasma cells in her bone marrow during her illness.

TABLE IV

DIFFERENTIAL COUNTS OF PLASMACYTES FROM BONE MARROW

Description	No. of Cases	Total Plasmacytes per 5,000 N.C.	Small Plasmacytes %	Medium Plasmacytes %	Large Plasmacytes %	Plasmablasts and Proplasmacytes %	Lymphoid Plasmacytes %	Plasmacyte Giant Cells %
Normal children	12	19.8	10.0	14.6	31.3	31.3	11.6	2.0
Acute rheumatic fever	22	170.0	11.4	18.0	30.9	31.1	8.7	2.1
Convalescent rheumatic fever	15	39.1	6.4	12.3	23.1	48.7	7.2	1.8
Inactive rheumatic fever	8	27.8	5.4	15.5	27.4	37.0	13.8	1.8
Active Sydenham's chorea	7	24.0	12.1	16.6	20.4	35.8	9.6	3.7
Convalescent Sydenham's chorea	6	24.8	16.5	16.9	15.7	35.5	7.6	0.4
Acute streptococcal pharyngitis	3	35.1	8.5	16.8	23.6	38.4	16.8	1.4
Convalescent streptococcal pharyngitis	6	60.0	8.8	12.1	22.1	37.7	16.0	3.8

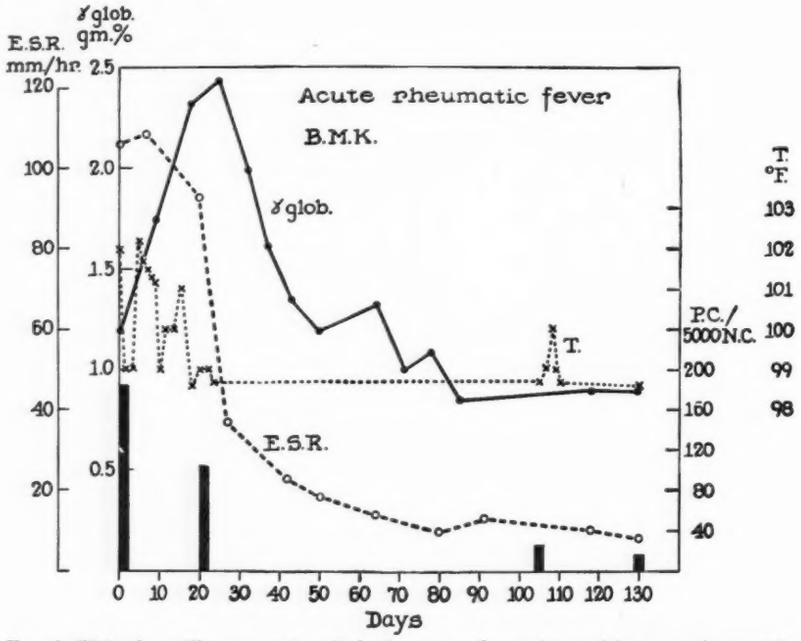

FIG. 3. This chart illustrates the clinical course of a patient with acute rheumatic fever. Note the relationship of the gamma globulin level (γ glob.) in the serum to the clinical activity of the disease as indicated by the temperature (T.) and erythrocyte sedimentation rate (E.S.R.). The relationship of the globulin level of the serum to the number of bone marrow plasmacytes (black bars) is clearly shown.

Initially the bone marrow plasmacyte count and the levels of serum gamma globulin were

both elevated. During the first two weeks of her rheumatic episode the gamma globulin continued to climb to higher levels, but during the third week its rate of increase tapered off. A bone marrow biopsy done at that time revealed that although the number of plasmacytes was

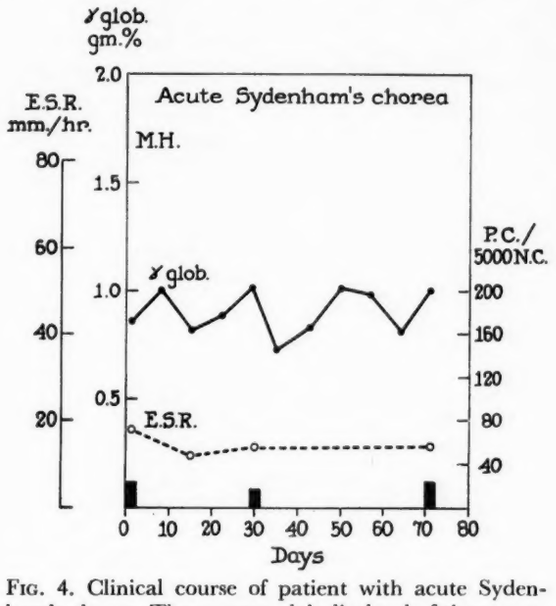

FIG. 4. Clinical course of patient with acute Sydenham's chorea. The gamma globulin level of the serum, the erythrocyte sedimentation rate and the plasmacyte count of the bone marrow remained within normal levels throughout the illness.

still elevated it had returned toward normal. With the progressive fall in the gamma globulins to normal levels during the succeeding three months there was noted a comparable return of the plasmacytes of the bone marrow to numbers within the normal range.

In striking contrast to this course is that of the patient illustrated in Figure 4. This seven year old girl was admitted to the University of Minnesota Hospitals with severe acute Sydenham's chorea. The choreiform movements were incapacitating on admission and the child was markedly dysphonic. In spite of the apparent severity of the chorea, she was afebrile and showed neither elevation of erythrocyte sedimentation rate nor leukocytosis. The plasmacytes of her bone marrow were both numerically and morphologically normal, and the serum gamma globulin level fell within the normal range.

No significant changes in either the numbers of bone marrow plasmacytes or the level of serum gamma globulins were seen at any time in spite of the striking clinical improvement wit-

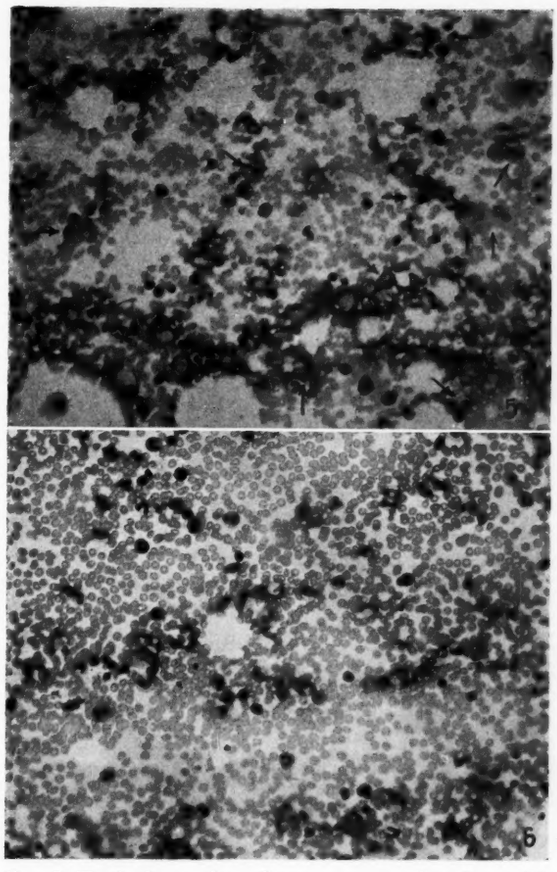

FIG. 5. Typical area from bone marrow of patient with active rheumatic fever. Note the marked plasmacytosis.
FIG. 6. Typical area from bone marrow of normal child. Note the absence of plasmacytes.

nessed during the period of hospitalization. Upon discharge from the hospital two and a half months after admission this child appeared to be completely well, with no evidence of rheumatic cardiac injury. The levels of serum gamma globulin and the numbers of bone marrow plasmacytes were no different at this time from comparable measurements obtained at the peak of her illness.

Figure 5 is a photomicrograph of a typical area from the bone marrow of a patient with rheumatic fever. This may be compared with a similar area from the bone marrow of a normal child. (Fig. 6.) The abundance of plasmacytes distinguishes the marrow of the rheumatic patient from that of the normal child of the same age. In each figure 150 to 200 nucleated marrow elements are illustrated. While at least ten plasmacytes can be identified in the illustra-

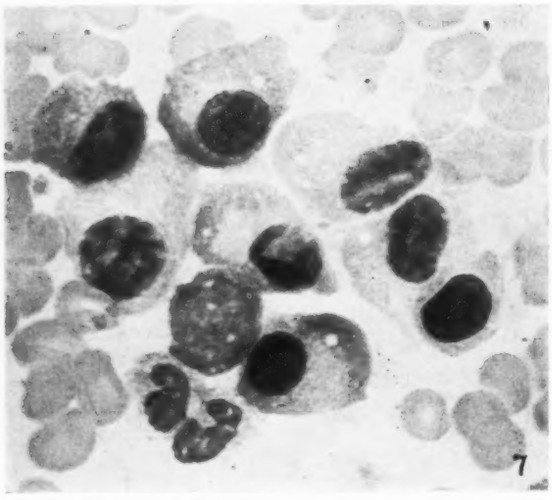

FIG. 7. Cluster of plasmacytes from the bone marrow of a patient with acute rheumatic fever. The morphologic characteristics of these developing plasmacytes may be compared to those of a developing normoblast.

tion of the marrow specimen from the rheumatic fever patient (Fig. 5), none are to be seen in the comparable normal sample.

In Figure 7 is shown a high power view of a cluster of plasmacytes taken from the marrow of a patient with acute rheumatic fever. Such accumulations of plasmacytes, which are not uncommon in the bone marrow of rheumatic patients, are rarely if ever seen in the bone marrows of normal children nor, in our experience, in the bone marrow of diseased children having normal levels of serum gamma globulin.

COMMENTS

It has been shown by the data presented above that patients with acute rheumatic fever regularly develop plasmacytosis of the bone marrow which disappears upon the return of the patient to rheumatic quiescence, and that this alteration of the bone marrow correlates well with the production of gamma globulin in this disease. Evidence presented by Anderson, Kunkel and McCarty[17] seems to indicate that in rheumatic fever, variations in serum gamma globulin reflect antibody formation against the streptococcus and its products. It would then seem likely that the bone marrow plasmacytosis which we have observed in rheumatic fever could best be explained as a morphologic expression of the response of the reticulo-endothelial tissues to the antigenic stimulation afforded by contact with the group A streptococcus and its products. To be sure, this plasmacytosis of the bone marrow in rheumatic fever is neither a specific reaction of the disease nor of the tissue being studied in this investigation but represents a more general phenomenon. Both early and recent literature attest to the widespread occurrence of plasmacytosis in human disease. Furthermore, in most instances plasmacytosis of the bone marrow has proved to be a reflection of comparable alterations in other organs of the reticulo-endothelial system. Study of the spleen, lymph nodes or diffuse lymphatic tissue would presumably reveal the same plasmacytic response indicated by our studies of the bone marrow in acute rheumatic fever.

The concept that plasmacytes play a special role in body defense mechanisms is an old one, rooted in an earlier speculative literature[23,24] and fostered by studies of Downey,[25] Sitko,[26] Huebschmann,[27] Allessio,[28] Mas y Magro,[29] Miller[30] and others. Beginning in 1937 with studies of agranulocytosis, Bing et al.[31,32] established a striking positive correlation between bone marrow plasmacytosis and hyperglobulinemia in a wide variety of diseases. Since Bing's work was begun, many others have supported the reality of this relationship on the basis of clinical studies.[33–36] Simultaneously with the initiation of the clinical investigation, Kolouch[37] began an experimental investigation of the relationship of plasmacyte formation and antibody production, and showed that immunization of rabbits with streptococcal antigens is regularly accompanied by the development of bone marrow and splenic plasmacytosis. With him, we[38] further demonstrated that, following anaphylactic shock of rabbits with either bacterial or simple protein antigens, the development of bone marrow plasmacytes from plasmablasts was correlated with the

presumed elaboration of specific antibodies. In agreement with Rohr it was shown that the precursor of the plasmacyte in the bone marrow was a derivative of the reticulum, and that plasmacytes developed in turn from this plasmablast by heteroplastic metamorphosis. The development of the mature plasmacyte was associated with the liberation of globulin and specific antibodies into the serum. Meanwhile, Bjorneboe and Gormsen[39] further linked plasmacytes to specific antibody production when they found that intensive, prolonged immunization with polyvalent pneumococcus vaccines produced both an unprecedented accumulation of antibodies in the serum of rabbits and a tremendous plasmacytosis in all tissues possessing reticular elements capable of mobilization. This work has since been confirmed in our laboratory. These workers further demonstrated that plasmacyte-rich tissues contain an abundance of specific antibodies.

Further studies in our laboratory[40–43] indicate that the local "secondary response" to an antigen with which an animal has had previous contact is characterized by a rapid and intense accumulation of plasmacytes in the inflammatory exudate. This plasmacytic reaction has been shown by Goddard[44] to be a characteristic of the Arthus reaction in loose connective tissue as well. Fagraeus[45] found that not only is the "secondary response" in the spleen productive of marked plasmacytosis and intensive antibody release *in vivo*, but that in tissue culture the plasmacyte-rich red pulp of the spleen produces a surprising amount of antibody after such stimulation. Ehrich and coworkers[46] have recently pointed out that evidence from study of antibody formation by lymph nodes[47,48] can now be interpreted as evidence for the plasmacyte theory of antibody production. This has been made possible by their recent studies showing that the peak production of antibody by lymph nodes corresponds precisely in time with the peak of the accumulation of plasmacytes and ribose nucleic acid in these structures while maximal lymphocytic proliferation and desoxyribose nucleic acid accumulation are not reached until antibody production is returning toward normal.

The experimental evidence linking the plasmacyte with antibody and gamma globulin production is rendered more credible by virtue of its agreement with data accumulated from clinical studies, particularly those indicating that the plasmacytic malignancies (multiple myeloma, plasma cell leukemia and diffuse plasma cell myelosis) are characterized by abnormal protein formation and not infrequently by marked hypergammaglobulinemia.[49]

The concept that antibody and gamma globulin are produced by plasmacytes is given further support by the close correlation observed in this study between plasmacytic development in the bone marrow and gamma globulin production. Of particular significance is the striking correlation between the slope of the curve of gamma globulin accumulations and the degree of plasmacytosis shown in Figure 3 and observed in other patients of this series.

In addition to giving support to the plasmacyte theory of antibody production our data further adds support to the concept that acute rheumatic fever follows in the wake of infection, presumably with group A streptococcus. The elevated levels of serum gamma globulin observed regularly during the acute phase of this disease followed by a return to normal levels during convalescence are certainly in keeping with this thesis, while the plasmacytosis of the bone marrow serves as a further indication of host response to antigenic stimulation in rheumatic fever. It would seem from these data that uncomplicated Sydenham's chorea differs markedly from acute rheumatic fever in respect to alterations of the bone marrow and serum gamma globulins. It is well known that many laboratory manifestations which regularly accompany other clinical manifestations of acute rheumatic fever are absent in chorea. We[50,51] have recently reported the occurrence of similar differences between chorea and acute rheumatic fever with respect to certain acute

phase protein reactions, and in the present study evidence of additional biochemical and morphologic differences between patients with chorea and those with acute rheumatic fever is presented. It would seem reasonable in the light of these findings to group data on patients with uncomplicated chorea separately from those obtained on patients with acute rheumatic fever when analyzing biochemical characteristics of these diseases, rather than to pool such results as is often done.[52,53]

It is of real interest that the bone marrow, and the reticulo-endothelial elements in particular, show no differences from the normal in patients with active and inactive chorea and those with inactive rheumatic fever. It is obvious that this study has not provided an explanation for the genetically determined susceptibility to rheumatic fever which characterizes these patients and members of their families.[54]

The greater production of antibodies against streptococcal products and the greater production of gamma globulin as well as the more intense bone marrow plasmacytosis in patients who develop rheumatic fever than in those who have streptococcal infection without sequelae pose important problems. Could this be evidence that an allergic or "secondary" response is involved in the pathogenesis of rheumatic fever, or is it merely evidence that rheumatic fever occurs more often following streptococcal infections giving strong antigenic stimulation? The findings here reported would be compatible with either hypothesis.

SUMMARY AND CONCLUSIONS

1. Quantitative studies of the bone marrow from normal children and from children in various stages of activity of rheumatic fever, chorea and streptococcal pharyngitis are reported and compared. Simultaneous studies of the levels of serum gamma globulins in these groups of children are also reported.

2. Plasmacytosis of the bone marrow was found to occur regularly in patients suffering from acute rheumatic fever and in patients convalescent from streptococcal pharyngitis, which is positively correlated with the increase in serum gamma globulin demonstrated in these diseases.

3. Normal numbers of bone marrow plasmacytes and normal levels of serum gamma globulin were found to be characteristic of normal children, of patients with inactive rheumatic fever and of patients with chorea.

4. The relationship of these findings to the plasmacyte theory of antibody production and to the streptococcal theory of the pathogenesis of rheumatic fever is discussed.

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Review

Cryptococcosis*

A Review with Special Reference to Apparent Association with Hodgkin's Disease

BENJAMIN R. GENDEL, M.D., MILTON ENDE, M.D. *and* STAFFORD L. NORMAN, M.D.
Memphis, Tennessee *Petersburg, Virginia* *Memphis, Tennessee*

CRYPTOCOCCOSIS is a systemic fungus infection caused by the Cryptococcus neoformans, a yeast-like organism also known as Torula histolytica. The infection shows a predilection for the central nervous system but may also involve the lungs and the skin. In general, it is considered to be a rare disease but in 1941 Binford[1] collected seventy-five cases from the literature and since then many additional cases have been reported. In addition to the many reports of single cases, Reeves and co-workers[2] reported six, and Cox and Tolhurst[3] thirteen patients with cryptococcal infection.

We have reviewed the literature since Binford's article and have found reports of eighty-eight additional cases.† To these we add two of our own to bring the total number of cases reported to 165. It should be obvious from the large number of recent articles that the condition is not as uncommon as is generally believed. Many cases are undoubtedly overlooked because the condition is not often considered in the differential diagnosis of infections of the central nervous system. In addition, as in our cases, the infection may complicate another disease and consequently diagnostic measures may not be sufficiently intensive, particularly when the fundamental condition is capable of producing central nervous system complications.

The causative organism, C. neoformans, is a budding fungus, similar to Blastomyces, but differs from this organism in its failure to produce mycelia on artificial culture media. The organism appears in the spinal fluid or other infected tissue as a spherical, thick-walled, yeast-like structure from 5 to 20 micra in diameter. Each yeast cell is surrounded by a well defined, clear, refractile capsule and budding forms may be found. The organism grows readily on the common culture media, including blood agar, glucose agar and Sabouraud's medium. For a more detailed description of the mycology of the organism several recent monographs may be consulted.[3,6]

Cryptococcal infections have been reported from all parts of the world. At one time the disease was called European blastomycosis to distinguish it from North American blastomycosis caused by Blastomyces dermatitidis. The disease is also described under the names of torulosis and cryptococcosis. Most of the cases have been reported from the United States but recently many cases have been described in South Africa[7–9] and Australia,[3,10–15] pointing to the widespread distribution of the condition. Several naturally occurring infections in

† Case 3 in the report of Tinney and Schmidt[4] is undoubtedly the same as Case 3 of Greening and Menville's[5] report. Consequently this case is included only once in our compilation.

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animals have been reported[16,17] but there has been no evidence to show that the disease has been transmitted from infected animals to man. In addition, transmission from man to man is not believed to occur. Cryptococci have been found free in nature and have been isolated from many plants, the bodies of insects, pickle brine and fermenting fruit juices. Benham[18] isolated cryptococci from the skin of normal individuals and compared these strains with other strains from patients with clinical disease and with cryptococci occurring free in nature. These latter non-human strains could be differentiated from the cryptococci of human origin by cultural, morphologic and serologic criteria. However, some of the cryptococci found on the skin were similar to strains capable of producing disease.

The disease has been described in all races. There is a predilection for males as demonstrated by involvement of seventy-one males and only thirty-seven females in a series collected by Voyles and Beck.[19] The occurrence in the newborn has recently been described by Neuhauser and Tucker.[20] These authors reported three premature infants who, within several weeks of birth, developed an illness which resembled neonatal toxoplasma infection in that intracerebral, punctate and confluent calcification was found on skull films. Evidence of mild hydrocephalus occurred accompanied by hepatosplenomegaly. The condition was characterized by early death. All patients were found to have diffuse infection with C. neoformans and two of the patients showed inflammatory and granulomatous lesions in the lungs. In their second case C. neoformans was grown from smears of the mother's endocervix. They suggest that the infection was acquired during delivery. The portal of entry is not definitely known but most observers believe that the organism usually gains access to the body by way of the respiratory tract. This is suggested by the presence of lesions in the lungs in a significant number of cases. Under other circumstances the organism may enter the body by way of the skin.[21]

Central Nervous System. Involvement of the central nervous system is by far the most common type of infection by C. neoformans. This may occur as a meningo-encephalitis alone or may be a part of a generalized infection. Greening and Menville[5] collected 107 cases from the literature and found that the central nervous system alone was involved in eighty-three and the central nervous system together with the lung in twenty cases. Freeman[22] described three types of involvement: (1) a meningeal type which is most common and is characterized by a diffuse granulomatous meningitis; (2) a perivascular form accompanied by small granulations or cysts in the cortex and (3) an embolic type with deeply placed lesions in the cortex. The clinical picture is that of a low-grade meningo-encephalitis and is characterized by signs and symptoms of increased intracranial pressure. Headache, drowsiness and vomiting are commonly noted. Physical findings are neck rigidity, opisthotonos, papilledema, amblyopia, diplopia, nystagmus and paralyses. Occasionally a focal collection of cryptococci may simulate a brain tumor and localizing signs may predominate.[23,24,25,25a] Cranial nerve and peripheral paralyses have been described. In some patients a psychosis may be the presenting difficulty. Cryptococcal infection in the newborn may manifest itself by multiple intracranial calcifications simulating toxoplasmic encephalomyelitis.[20]

The spinal fluid is found to be under increased pressure. It is usually clear and the cell count varies from normal to several thousand with an average of 200 to 800 cells. The predominating cell is the lymphocyte although occasionally a neutrophilic pleocytosis may occur.[7,26,27] One cause for mistaken diagnosis is failure to recognize the cryptococci. These organisms are frequently confused with lymphocytes or red cells. The finding of budding forms should immediately indicate the proper diagnosis. A most helpful technic is the mixture of the spinal fluid sediment with a diluted India

ink solution. This causes the organisms to stand out characteristically. (Fig. 1.) Stained preparations are also of assistance but the diagnosis depends chiefly upon culture of the organism. Fortunately the organism grows readily on ordinary culture media. However, growth may be slow and many cases are probably overlooked because of failure to observe the cultures long enough. A period of four to eight days should suffice to produce a positive culture. A single negative culture does not rule out the possibility of central nervous system cryptococcosis because not infrequently in well authenticated cases of the disease a negative culture was obtained interposed between a number of positive ones. This was demonstrated in our Case I. Knowledge of this fact should also be kept in mind in evaluating therapy.

TABLE I

SUMMARY OF SPINAL FLUID FINDINGS AND TREATMENT IN CASE I

Date	White Cell Count (per cu. mm.)	Differential	Culture	Sugar (mg. %)	Protein (mg. %)	Kolmer	Treatment
5/4/48	102	L 60 P 40	Sterile for pyogens	40			
5/11/48	104	L 60 P 40	Sterile for pyogens			Pos. 0.125 cc.	May 5–May 28, 1948, penicillin 50,000 units q. 3 hr.; total dose 9,000,000 units
7/27/48	68	L 41 P 59	C. neoformans	28	100	Pos. 0.25 cc.	
8/6/48			C. neoformans	28	112		
8/17/48	35	?	Sterile		132		Aug. 17–Aug. 30, 1948, sulfadiazine; blood levels 10–15 mg. per cent
9/1/48	56	L 15 P 85	C. neoformans		126		
9/29/48	90	L 12 P 88	C. neoformans	35	140		Sept. 15–Oct. 11, 1948, massive sulfadiazine; blood levels 15–22 mg. per cent
10/8/48	80	P 100	Sterile	36	120	Pos. 0.25 cc.	
11/15/48	74	L 20 P 80	Sterile	40	176	Pos. 0.5 cc. Dbtfl. 0.25 cc.	Nov. 29–Dec. 7, 1948, penicillin 1,000,000 units q. 2 hr.; daily dose 12.0 M units
12/2/48	54	L 37 P 63	C. neoformans	30	180	Pos. 1.0 cc.	
12/6/48	28	L 35 P 65	C. neoformans	37	80	Dbtfl. 0.5 cc.	
12/14/48	72	L 25 P 75	C. neoformans	25	123		Dec. 7–Dec. 29, 1948, penicillin 1,000,000 units q. 3 hr.; daily dose 8.0 M units
12/21/48	51	L 30 P 70	C. neoformans	28	154	Pos. 0.25 cc.	
12/30/48	54	L 39 P 61	C. neoformans	42	204		
							Jan. 8–Jan. 18, 1949, streptomycin 2.0 gm. daily intramuscularly

CASE I. The patient was a sixty-two year old colored man who was admitted to the hospital on May 4, 1948. He was disoriented and incoherent so that an adequate history could not be obtained. Apparently he had been well until three weeks prior to admission at which time he developed increasing debility, incoherence and incontinence of urine and feces. A vague history was obtained of a penile lesion in 1918 and a course of weekly injections but the accuracy of this information was doubtful because of the patient's mental status.

The patient was a cachectic colored male. His temperature was 101.4°F., blood pressure was 130/70. The pupils were irregular and did not respond to light. The ocular fundi revealed no abnormalities. The heart, lungs and abdomen were normal. The patellar and Achilles tendon reflexes were absent bilaterally.

The admission blood count revealed a red cell count of 3.6 million; white blood cells 9,100; hemoglobin was 11.1 gm. per cent. The differential revealed 85 per cent polymorphonuclear cells, 8 per cent lymphocytes, 6 per cent monocytes and 1 per cent basophiles. The urine was

normal except for a faint trace of albumin. NPN was 26 mg. per cent. The Kahn test was positive, 16 units; the Kolmer-Wassermann reaction was positive, 64 units; the cardiolipin flocculation and complement fixation tests were positive, 16 and 128 units, respectively. The spinal fluid findings are summarized in Table I.

A diagnosis of central nervous system syphilis was made and the patient was treated with 50,000 units of penicillin every three hours for a total of 9,000,000 units. There was no significant change in his condition. In June, 1948, he developed an acute episode characterized by stupor, dyspnea, orthopnea, fever and a fall in blood pressure to 65/45. Symptomatic treatment with digitalis, oxygen and glucose infusions resulted in gradual improvement. On July 27, 1948, the patient developed opisthotonos and neck rigidity. A spinal tap was performed; culture of the fluid revealed cryptococci. Sensitivity studies showed that the organisms were not inhibited by either 1,000 units of penicillin per cc., 50 units of streptothrycin per cc. or 100 units of streptomycin per cc. The patient had an intermittent, usually low-grade fever throughout his hospital stay. This was uninfluenced by any type of therapy. The various forms of chemotherapy utilized are outlined in Table I. The patient died on January 18, 1949. Permission for autopsy could not be obtained.

Lungs. Coincident involvement of the lungs has been noted in about 20 per cent of the cases. The recent careful studies of Cox and Tolhurst[3] indicate that this incidence may be too low. They found complicating pulmonary disease in eight of thirteen cases. In six the lesion was found to be definitely cryptococcal in origin, in two others this was uncertain. Involvement of the lung alone is rare but has been reported by several observers. Sheppe[28] is credited with the description of the first case of solitary pulmonary involvement in 1924. However, there is some doubt about this case. Stiffness of the neck was noted but unfortunately a spinal tap was not done. At postmortem examination cryptococci were found in a bronchopneumonic area in the right lung; the brain was not examined. Solitary involvement of the lung was reported by Hardaway and Crawford,[29] Greening and Menville[5] and Moody.[30] Other cases have been reported with primary pulmonary involvement but with subsequent spread to the central nervous system.[31,32]

The pulmonary lesions occur in two forms, either as gelatinous masses or as fibrotic granulomatous lesions. The first group consists of foci of cryptococci which have undergone massive proliferation into a gelatinous mass surrounded by little reaction in the tissues of the host. The second type reveals a paucity of organisms which have produced a granuloma and fibrosis without visible gelatinous lesions.

Skin. Lesions of the skin are usually part of a disseminated infection with C. neoformans. The most common skin manifestation is an acneiform eruption. Occasionally this type of lesion may ulcerate and exude a purulent material which contains the organisms. Wile[33] reported the occurrence of bluish-black purpuric lesions having a board-like consistency and occurring on the lower extremities. Biopsy of the lesions disclosed the fungi. Johns and Attaway[21] reported a patient in whom a granuloma developed at the site of a razor-cut. Cryptococci were isolated from the lesion. The patient later died of meningitis.

Localized Involvement. Occasionally localized involvement may occur. This has been reported in muscle,[34] the soft tissues of the pelvis,[35] the tongue,[36] nasopharynx[37,38] and elsewhere. Table II lists the pertinent data concerning the reported cases. It is significant that despite the grave prognosis in other forms of cryptococcosis, all of this group recovered despite a variety of forms of treatment. The principal danger is dissemination of the infection.

Disseminated Involvement. In some cases the infection may be generalized and involve a variety of organs. This is noted particularly at postmortem examination. The kidneys are not infrequently involved and cryptococci have been found in the glomeruli. Organisms have been cultured from the urine.[22,42] We were unsuccessful in attempts to culture cryptococci from the urine of our first patient. The liver, spleen, thyroid and

testes have all been involved as part of a generalized process. Symptoms related to such individual organ involvement are unusual. Lesions of the adrenals have been described and adrenal insufficiency has been attributed to the infection.[43] Bone lesions

TABLE II

REPORTED CASES OF LOCALIZED INFECTION WITH C. NEOFORMANS

Author	Race	Sex	Age Yr.	Type of Lesion	Location	Duration	Treatment	Result
Brewer and Wood[34]	W	M	20	Abscesses of muscle	Adjacent to vertebrae $T_{2,3,4}$, $L_{1,2,3}$	3 mo.	Wide dissection and drainage	Healed in 2–3 wk.; well one year later
McGehee and Michelson[35]	N	F	26	Abscess	Soft tissue of pelvis and inguinal region	4 mo.	Incision and drainage; iodides	Healed; well 9 mo. later
Alvarez[36]	W	M	30	Superficial, red, grass-like filaments	Tongue	?	Arsphenamine and quinine	Healed in 1 mo.
Jones[37]	W	M	34	Granulomatous nodules and ulceration	Nasopharynx	1½ yr.	Cauterization; iodides; ultra-violet; x-ray	Slow healing; considered arrested after 1½ yr.
Case 1	W	F	12	Abscess	Rt. orbit and sinuses	Few days	Drainage of abscess; iodides; x-ray	Healed; well on 7 yr. follow-up
Case 2	W	M	26	Ulcerative lesions	Left tonsil and soft palate	4 mo.	Iodides and x-ray	Healed; well on 2 yr. follow-up
Gill[38] Case 3	W	M	26	Ulcerative lesions	Pharynx and epipharynx	2 yr.	Iodides and x-ray	Ulcers healed but cultures still pos. after 21 mo.; patient still complained of painful throat
Case 4	W	F	27	Ulcerative lesion	Hard palate	Few days	Iodides and x-ray	Healed; well on 2 yr. follow-up
Kessel and Holtzwart[41]	W	M	38	Abscesses	Both breasts and left knee joint	?	X-ray to breast; arthrotomy; iodides; amputation of left leg	Healed
Burger and Morton[40]	W	F	41	Soft tissue mass	Right thigh	6 mo.	Amputation right thigh at hip joint	Healed; well 3 yr. later
Dienst[39]	N	F	22	Encapsulated tumor	Rt. post. chest wall	5 wk.	X-ray; iodides; removal of mass	Healed after 6 wk.

are rare although the ilium was involved in Cleland's case[13] and the knee joint in the case reported by Kessel and Holtzwart.[41] The infrequent occurrence of bone lesions serves as a contrast to infection with B. dermatitidis and C. immitis.

Association with Hodgkin's Disease. Although reference is made in the literature to the association of these two diseases, this knowledge is not widespread. In reviewing the reported cases of cryptococcosis we have found thirteen cases in which coincident Hodgkin's disease was thought to be present. To this group we add one case of our own, making a total of fourteen cases with this interesting association, an incidence of 8.5 per cent of all reported cases of cryptococcosis.

CASE II. A thirty-nine year old white male, life insurance salesman, was admitted to the hospital on July 23, 1948. The patient stated that in October, 1944, he developed swelling of the right axillary lymph nodes. Subsequently enlargement of the inguinal and cervical nodes developed. In June, 1947, a biopsy of a cervical lymph node and a skin lesion of the right scapular region revealed Hodgkin's disease in both tissues. The patient received a course of x-ray irradiation in July, 1947, and in November, 1947. Regression of nodes and symptomatic improvement followed. In April, 1948, swelling of the abdomen, shortness of breath and edema of the feet and ankles were noted. A third course of x-ray therapy at this time did not result in improvement. In early July, 1948, abdominal and thoracic paracenteses resulted in the removal of chylous fluid. Recurrence of swelling and dyspnea necessitated a second paracentesis two weeks later. Numbness of the right leg and muscular weakness of the right arm developed.

The patient was a severely ill white man who was dyspneic and orthopneic. The significant positive findings were the absence of breath sounds over the lower three-fourths of the right lung accompanied by dullness to flatness on percussion. The abdomen was markedly distended with fluid. An indefinite mass, believed to be spleen, was palpable in the left upper

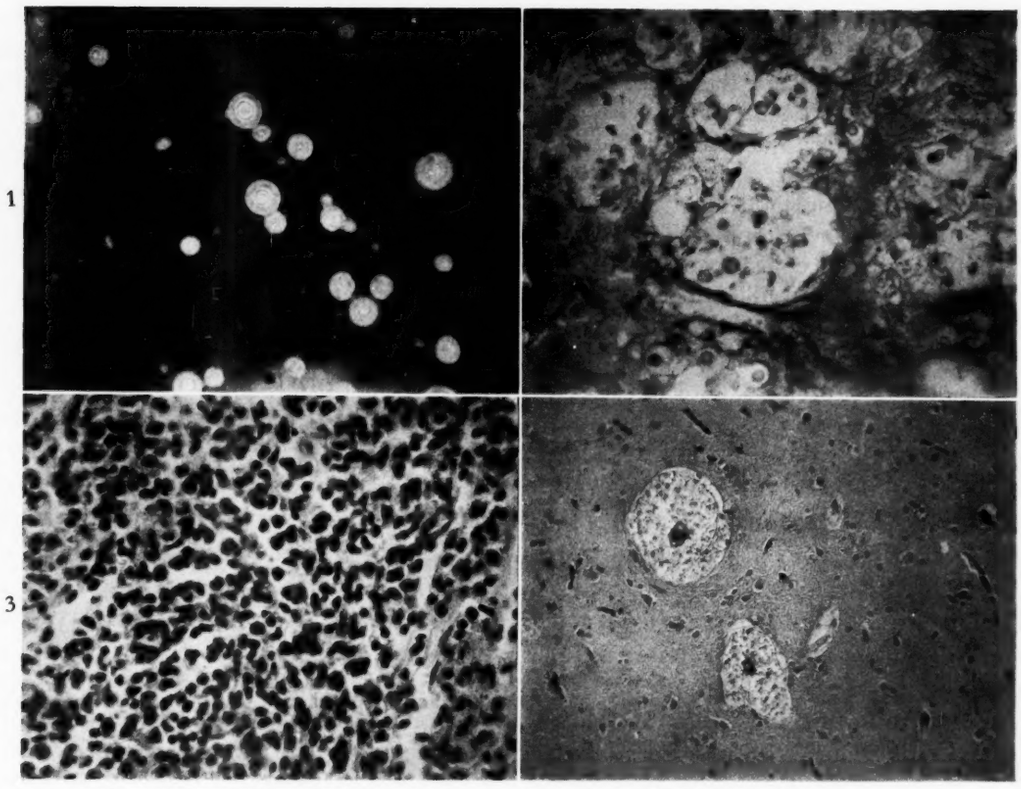
quadrant of the abdomen. A right inguinal hernia was present. He was unable to extend the right wrist, flex the forearm or raise the arm above the head. Deep reflexes were diminished generally but especially in the right upper extremity. At the base of the neck on both sides hard, discrete, non-tender nodes were palpated. These measured about 1 by 1 cm. on the right and about 2.5 by 1 cm. on the left. The entire neck was markedly indurated.

The blood count on admission showed a red cell count of 4.4 million; white cell count was 7,300; hemoglobin was 12.6 gm. per cent. The differential count showed 81 per cent polymorphonuclear cells, 16 per cent lymphocytes and 3 per cent monocytes. The urine was normal. X-ray of the chest revealed a hydropneumothorax on the right with complete collapse of the lung. A small pleural effusion was noted on the left. A metastatic x-ray survey revealed no bone involvement.

The severe and progressive dyspnea and abdominal swelling made it necessary to perform repeated abdominal and thoracic paracenteses. The fluid removed had the appearance of chyle and settled out into two layers, the upper layer being creamy in appearance. Following removal of the abdominal fluid an enlarged, irregular, hard spleen could be palpated easily in the left upper quadrant. The patient was given a course of nitrogen mustard, 0.1 mg. per kg. of body weight of methyl-bis (β-chloroethyl) amine hydrochloride, starting on July 28, 1948, and continuing for four successive days (total dose 22.0 mg.). There was no significant change in his condition. On August 17, 1948, the patient complained of moderate headache which had been present for the preceding few days. No neck rigidity or other significant new findings were noted. However, a lumbar puncture was performed. The pressure was 240 to 260 mm. of water and the fluid was clear. The headache improved for a few days following this procedure. Study of the spinal fluid revealed 10 cells per cu. mm., 7 polymorphonuclears and 3 lymphocytes. Sugar was 40 mg. per cent. Protein was 20 mg. per cent and chlorides 652 mg. per cent. No organisms were noted on direct smear but ten days later a culture was reported positive for C. neoformans. A course of x-ray irradiation (750 r) had been given to the anterior mediastinum from August 19 to August 24, 1948. The patient's course was progressively downhill despite symptomatic treatment. Headache and gradually increasing stupor developed and following the report of the positive spinal fluid culture, treatment with sulfadiazine 5.0 gm. intravenously followed by 1.0 gm. intravenously every six hours was instituted. Stiffness of the neck and a positive Babinski on the right developed. The patient developed opisthotonos and expired on August 31, 1948. A review of the slides of the previous biopsy of lymph node and skin confirmed the presence of Hodgkin's disease.

The significant gross findings at autopsy were the presence of chylous effusions into both pleural cavities. The left contained 2,000 cc. and the right 3,500 cc. of fluid. Both lungs were collapsed, the right weighed 280 and the left 500 gm. Many soft elevated yellow nodules were present on the parietal pleura along the intercostal muscles and ribs. These varied in size, the largest measuring 1 cm. in diameter. Many petechial hemorrhages were noted. The abdomen contained 4,200 cc. of chylous fluid. Many small tumor nodules were scattered throughout the mesentery and omentum. There was a large mass in the mesentery of the small intestine measuring 6 by 6 by 4 cm. Loops of small intestine were matted together by fibrinous adhesions. The thoracic duct and cisterna chyli could not be identified because of complete replacement by tumor tissue. The spleen weighed 450 gm. and was nodular. This organ was almost completely replaced by tumor nodules measuring 1 to 6 cm. in diameter. The liver and gallbladder weighed 1,450 gm. The pancreas was surrounded by tumor tissue, which did not extend into the parenchyma except possibly in the region of the tail. The lymph nodes were generally small and fibrotic. A node at the base of the subclavian vein measured 4 by 2.5 by 1 cm. The intestinal tract was encircled by masses of tumor tissue in the region of the jejunum. A large tumor mass surrounded the descending colon, the rectum and ureter. The brain weighed 1,390 gm. Gelatinous material was attached to the dura adjacent to and extending into the longitudinal fissure.

Postmortem bacteriologic studies showed a growth of C. neoformans from the spinal fluid and from the chylous fluid in the thorax. (Fig. 1.) The abdominal chylous fluid was negative for organisms. Chemical studies of the chest fluid showed: total lipids 830 mg. per cent, fatty acids 43 mg. per cent, cholesterol 98 mg. per cent and cholesterol esters 51 mg. per cent. The analysis



FIGS. 1 to 4. Case II. Figure 1, India ink preparation of spinal fluid obtained at autopsy showing typical cryptococci including many budding forms. Figure 2, lung section showing organisms within small cystic spaces. The lack of inflammatory reaction is noteworthy (aniline blue stain). Figure 3, lymph node showing pleomorphic cytology with hyperchromatic nuclei in many of the cells, suggestive of a malignant lymphoma but not characteristic of Hodgkin's disease. Figure 4, brain section demonstrating markedly dilated perivascular spaces containing cryptococci (aniline blue stain).

of the abdominal fluid was approximately the same except that the total lipids were 940 mg. per cent.

Microscopic examination showed areas of cystic degeneration of the lower lobe of the left lung. These spaces contained small round clear bodies with a double refractile capsule, which measured 12 micra in diameter. (Fig. 2.) The organisms were also seen in the surrounding alveoli. When stained with aniline blue stain, they were much more prominent than with routine hematoxylin eosin stains. Similar areas were also found in the right lower lobe. The hilar nodes showed a distortion of the architecture and replacement by pleomorphic tumor cells with irregular hyperchromatic nuclei. (Fig. 3.) There was hyperplasia of the reticuloendothelial cells and distention of the lymph sinusoids which contained macrophages and chronic inflammatory cells. There was also cystic degeneration similar to that seen in the lung. In these areas many binucleate cells contained the yeast organisms. The periaortic nodes showed cystic areas which contained organisms. The normal structure was also replaced by pleomorphic tumor cells and hyalinized fibrosis. The tumor cells were small although several were large, irregular and contained two or more nuclei. The spleen showed replacement by cells similar to those noted in the periaortic nodes but with less tendency to pleomorphism. Necrotic granulomatous areas were present which contained cryptococci. The connective tissue surrounding the pancreas was infiltrated by tumor cells as noted above. The adrenals, the small intestine and mesentery were likewise infiltrated by tumor cells and yeast-like organisms could also be found in the latter two. The subclavian lymph node showed destruction of the architecture and replacement by tumor tissue which also showed hyaline fibrosis. Many of the cells contained yeasts. The brain showed cystic areas in the dura which contained cryptococci. Sections from the frontal cortex, parietal cortex, corpus striatum and cerebellum showed degenerative areas containing organisms. The

perivascular spaces of Hiss were dilated and contained cryptococci. (Fig. 4.) The pia of all sections showed edema and the presence of organisms. The opinion of the pathologist was that the patient had cryptococcosis involving the brain, meninges, lungs, spleen, mesentery, hilar, subclavian and aortic lymph nodes. In addition there was also a lymphoblastoma involving the pancreas, adrenals, spleen, intestine, subclavian, abdominal and hilar nodes.

The co-existence of Hodgkin's disease and cryptococcosis was first reported by Freeman and Weidman[44] in 1923. They described a thirty-nine year old man who had lymph node enlargement for some years. Five years previously a biopsy of one of the nodes revealed Hodgkin's disease. Following x-ray therapy the nodes regressed. Subsequently the patient died of central nervous system cryptococcosis after an acute illness lasting about a month. At autopsy the spleen, the right axillary and the mesenteric nodes were enlarged. The microscopic appearance was no longer considered characteristic of Hodgkin's disease. A similar observation was reported in 1930 by Smith and Crawford.[45] Their patient, a thirty-one year old woman, developed a mass over the left scapula. The histologic appearance of the specimen removed at biopsy was suggestive of Hodgkin's disease. The tumor mass was radiosensitive and disappeared following irradiation. About a year later the patient developed central nervous system complications manifested by diplopia, strabismus, bilateral sixth nerve palsy and a right seventh nerve paresis. The illness progressed to eventual death in six months. No evidence of the original scapular tumor was found. The brain, spinal cord and chest showed infection with cryptococci. Lesions suggestive of Hodgkin's disease were found in tissue removed from the posterior mediastinum. These first two cases showed certain atypical features and following autopsy because of change from the original histologic picture of Hodgkin's disease and the finding of cryptococci they were considered to represent cryptococcal infection simulating Hodgkin's disease. Similarly, at autopsy our patient no longer showed histologic lesions typical of Hodgkin's disease although the picture resembled a lymphosarcoma. A similar transition in histologic pattern has been previously reported during the course of Hodgkin's disease not complicated by cryptococcosis.[45a]

In 1934 two cases were reported which left little doubt about the co-existence of the two diseases. The case of a thirty-one year old man was presented at the Massachusetts General Hospital.[46] This patient developed a swelling in the neck and a biopsy showed Hodgkin's disease. He was treated by irradiation and the glands receded. About a year later, following a trauma to his head, he developed headache and stiffness of the neck. The cervical lymph nodes were found to be enlarged again and the spleen was palpable. Cryptococci were found in the spinal fluid. Autopsy revealed a cryptococcal meningitis with concomitant involvement of the kidney, adrenal and lungs. The spleen, the bronchial and retroperitoneal glands showed the picture of Hodgkin's disease. About the same time Fitchett and Weidman[47] described an eighteen year old Negro who died thirteen days after admission to the hospital. Glandular enlargement in the axillae, neck and inguinal regions had been noted for the preceding three years. Headache and central nervous system signs began three months prior to death. Disseminated cryptococcal infection was found at autopsy. The spleen was enlarged and showed a picture suggestive of Hodgkin's disease. Cryptococci could also be found in the spleen. These authors bring up the possibility that the C. neoformans might be one of several organisms which may induce the histologic picture of Hodgkin's disease. Burger and Morton[40] reported another patient with Hodgkin's disease complicated by cryptococcosis. They were also of the opinion that this infection could produce granulomas which could closely simulate Hodgkin's disease. Cox and Tolhurst[3] reported a similar patient and called attention to a report by Heine and co-workers[48] of a patient with Hodgkin's disease compli-

TABLE III

SUMMARY OF DATA ON PATIENTS WITH COINCIDENT CRYPTOCOCCOSIS AND HODGKIN'S DISEASE

Case	Author and Year	Sex	Age	Diagnosis of Hodgkin's	Result of X-ray Treatment	Diagnosis of Cryptococcosis	Organs Involved by Hodgkin's	Organs Involved by Cryptococcosis	Review of Previous Biopsy
1.	Freeman and Weidman[44] 1923	M	39	Biopsy of lymph node five years previously	Lymph nodes reduced in size	Made by lumbar puncture	Spleen; axillary, and mesenteric lymph nodes	Central nervous system	Cryptococci not found
2.	Smith and Crawford[45] 1930	F	31	Biopsy of mass over scapula 17 months prior to death	Mass disappeared	Made at post-mortem	Swelling over scapula	Central nervous system; lymph nodes	Cryptococci found
3.	Fitchett and Weidman[47] 1934	M	18	Biopsy of left axillary lymph node	None given	Made by lumbar puncture	Spleen; lymph nodes	Central nervous system; kidneys; pancreas; spleen; lymph nodes	Not mentioned
4.	Cabot Case[48] 1934	M	31	Biopsy of cervical lymph node one year before death	Swelling in neck subsided	Made by lumbar puncture	Spleen; bronchial cervical and retroperitoneal nodes	Central nervous system; kidneys; lungs; adrenals	Not mentioned
5.	Wile[33] 1935	M	17	Biopsy of cervical lymph node 4½ years before death	Not mentioned	Made at post-mortem	Not mentioned	Central nervous system; skin; generalized	Cryptococci found
6.	Owen[49] 1940	M	33	Biopsy of cervical lymph node 5½ years before death	Decrease in size of lymph nodes	Made by lumbar puncture	Lymph nodes	Central nervous system; cervical, mediastinal and mesenteric lymph nodes; generalized	Cryptococci found
7.	Heine, Lauer and Mumme[48] 1940	M	29	Biopsy of cervical lymph node 1½ yr. previously	?	Made by lumbar puncture	?	Central nervous system; lymph nodes; spleen; lungs; and liver	?
8.	Warvi and Rawson[50] 1942	M	39	Biopsy of cervical lymph node five mo. before death	Lymph nodes responded to treatment; cerebral symptoms improved after radiation to head	Made at post-mortem	Cervical, supraclavicular, axillary and inguinal nodes; liver; spleen; bone marrow	Central nervous system; lungs	Not mentioned
9.	Burger and Morton[40] 1944	M	36	Biopsy of cervical lymph nodes 3 mo. before death	Regression in size of nodes	Made at post-mortem	Liver; lymph nodes	Heart; lungs; kidneys; adrenals; mediastinal, axillary and inguinal lymph nodes; permission not granted to examine central nervous system	Cryptococci not found
10.	Tinney and Schmidt[4] 1944	M	23	Biopsy of cervical lymph node four yr. before death, two additional biopsies confirmed the diagnosis	Responded to radiation on two occasions	Made at post-mortem	Thymus; spleen; lungs; liver; adrenals; prostate; testes; thyroid	Thymus; spleen; lungs; liver; adrenals; prostate; testes; thyroid	Not mentioned
11.	Cohen[42] 1944	F	36	Biopsy of cervical lymph node	Nodes responded to x-ray	Made by lumbar puncture	Thoracic, cervical and abdominal nodes	Central nervous system	Not mentioned
12.	Cox and Tolhurst[3] 1946	M	62	Made at post-mortem	None given	Made at post-mortem	Lymph nodes; spleen; liver	Central nervous system; lungs	
13.	Debre and coworkers[51] 1946	F	12	Biopsy of cervical lymph node 3 mo. earlier	Clinical improvement	Made by lumbar puncture	Spleen; liver	Central nervous system; generalized	Not mentioned
14.	Author's case 1948	M	39	Biopsy axillary lymph node and skin one yr. earlier	Regression of lymph nodes and clearing of skin; good response to two courses of treatment	Made by lumbar puncture	Lymph nodes; spleen; pancreas; adrenals involved by a malignant lymphoma not typical of Hodgkin's	Central nervous system; lungs; spleen; mesenteric lymph nodes	No organisms found

cated by blastomycosis. They were of the opinion that Heine's case was really one of cryptococcosis and consequently it is included in our review. Table III summarizes the essential data concerning the previously mentioned cases and the others we have found in the literature.

The diagnosis of Hodgkin's disease was made in thirteen cases prior to the diagnosis of cryptococcosis. The one exception was the patient reported by Cox and Tolhurst[3] who was admitted to the hospital in a moribund condition. The diagnosis of both conditions was made at autopsy. It is interesting to note that ten of the patients received x-ray therapy and in each instance there was a regression of swelling and symptomatic remission. In this connection Owen[49] found that x-ray irradiation of cultures of C. neoformans did not affect the organisms. A review of the original biopsy slide was done in six cases including our own. In three of the cases cryptococci were found[33,45,49] and in the other three, including our case, the organisms could not be found.[40,44]

In addition, Mallory[52] stated that he had seen four other cases of coincident lymphoblastoma and cryptococcal infection. He also referred to an unpublished manuscript which reported about eighty cases of cryptococcal infection. In about a dozen the two diseases were found together. Jackson and Parker[53] mentioned a patient with Hodgkin's disease who died of cryptococcal meningitis. These cases have not been included in our tabulation for lack of sufficient data. Magruder[54] reported a patient with lymphatic leukemia of four years' duration who developed a terminal cryptococcal infection. The significance of this observation is uncertain.

The association of these two uncommon diseases occurs much too frequently to be simply a matter of chance. The explanation is not clear. However, it is interesting to speculate concerning two possibilities which suggest themselves. Several authors have offered the possibility that infection with cryptococci might occasionally produce a tissue reaction which is similar to that of Hodgkin's disease. Forbus[55] failed to recover fungus organisms of any kind in cases of Hodgkin's disease and it is doubtful that cryptococci are of much significance in the etiology of this disease. We found no evidence of cryptococcal infection in about thirty other patients with Hodgkin's disease. Eight of these patients had evidence of nervous system involvement and were studied in greater detail. Many micro-organisms have been isolated from patients with Hodgkin's disease. Diphtheroids, avian tubercle bacilli and brucella among others have received major attention. Desjardins[56] called attention to the frequency of many infections in patients with Hodgkin's disease and suggested that chronic infection of any kind was the factor immediately responsible for the malignant hyperplasia of the lymphoid structures. According to this viewpoint Hodgkin's disease should be considered as a syndrome produced by a variety of infecting agents, including C. neoformans. Cox and Tolhurst[3] noted that the lymph glands were frequently involved in experimental cryptococcal infection. This included not only the regional glands draining the inoculation site but also those in remote regions. The nodes were crowded with cryptococci. Fibrosis, giant cells and epitheloid cells were also found. However, despite this, there were no instances in which the histologic picture simulated Hodgkin's disease. It should be noted that a review of the original biopsy slide in three cases of cryptococcosis showed the presence of the fungus which was overlooked at first. None of this evidence can be considered conclusive, and it is not yet established that infection by C. neoformans can produce Hodgkin's disease.

An alternative explanation for the association of the two diseases is that patients with Hodgkin's disease are prone to develop a variety of infections. Dubin[57] recently called attention to the poor immunologic response in patients with Hodgkin's disease. He believed this poor immunologic response could explain the failure to react to tuberculin, the decreased incidence of positive serologic

reactions for syphilis and the increased tendency to the development of infection in patients suffering from Hodgkin's disease. In this connection Benham[18] showed that cryptococci can be recovered from the skin of normal individuals and that these organisms are similar to the pathogenic cryptococci of human disease. With this in mind, it is possible that the poor resistance of patients with Hodgkin's disease permits their own cryptococci to obtain a foothold and produce clinical disease.

Prognosis. Cryptococcosis is a serious disease and involvement of the central nervous system is nearly always fatal. The one notable exception is the case reported by Marshall and Teed.[58] These authors described a nine year old girl who recovered following mastoidectomy and the administration of sulfadiazine. In other instances patients who had not died of their disease at the time of the report continued to show evidence of persistent infection. Reeves and co-workers[2] reported a patient who was alive about eight years after the onset of her illness.[19] Their patient also received sulfonamides. In contradistinction to the poor prognosis in central nervous system cryptococcosis, the outlook in localized forms is much better, provided spread of the infection to the central nervous system does not occur. Localized cases with recovery are reported in Table II. Occasionally the disease may begin as a localized form and later disseminate with fatal outcome. The localized pulmonary form also has a better prognosis.[5]

Treatment. The treatment of this disease is unsatisfactory. The hope engendered by Marshall and Teed's[58] report of recovery following administration of sulfadiazine has not been confirmed by subsequent observers.[19,31,32,59–61] Our experience with sulfadiazine was poor despite the attainment of high blood levels in our first patient. Animal experiments showed no beneficial effect of the administration of sulfadiazine, potassium iodide or combinations of the two drugs.[62] A similar lack of effect has been noted with penicillin.[59] The use of massive doses of penicillin in our first patient also failed to clear the spinal fluid of organisms. Beck and Muntz[64] reported animal experiments with streptomycin and found suggestive evidence that this drug might prove of value. In light of this, clinical trial of streptomycin is certainly justified. Unfortunately, we could not evaluate the results of the use of streptomycin in our patient.

SUMMARY

The literature on cryptococcosis has been reviewed and 165 reported cases have been collected including two cases of our own. The clinical aspects of the disease have been discussed. Fourteen of these patients have had coincident Hodgkin's disease. The possible mechanisms for this association have been mentioned.

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Seminars on Renal Physiology

Acid-base Regulation by the Kidneys*

ROBERT F. PITTS, M.D.
New York, New York

According to Macallum[1] our protovertebrate ancestors evolved in the sea at a time when its salinity was roughly one-third its present value. An abundance of dissolved sodium bicarbonate and carbon dioxide created a blandly alkaline environment eminently favorable for the functioning of their body cells. These animals could drink freely of the fluid surrounding them, pump it through their vascular systems, allow it to permeate their tissues, secrete it into their body cavities and then propel it and its dissolved wastes to the outside through simple ciliated tubes. Availability of salt, water and alkali constituted no problem for these animals, for the whole of the ocean containing these components in optimum proportions was at their disposal. The permeability relationships of their cell membranes and the intracellular ionic environment of their vital enzyme systems had been established in the presence of a faintly alkaline saline medium. When they migrated first into fresh water and later onto dry land, it was imperative that they enclose within their integument a bit of this fluid medium and develop means of defending it against changes in composition and reaction.

Sodium chloride and sodium bicarbonate are the major salts of the blood plasma and interstitial fluid of man and no doubt of the Cambrian ocean which his forebears deserted some 500 million years ago. These fluids are alkaline due to the sodium bicarbonate which they contain. The degree of alkalinity of all is tempered or buffered by a relatively fixed concentration of carbonic acid (dissolved carbon dioxide). The reaction of the Cambrian ocean must have been stabilized by its large volume and by the fact that the exchanges of carbonic acid and bicarbonate between the heavily charged atmosphere, the mineral-bearing waters and the sediment beds which formed the great rock strata of this era were in equilibrium. In contrast, the reaction of the blood plasma and interstitial fluid of the body must of necessity be actively and continuously stabilized by respiratory and renal homeostatic mechanisms which preserve, respectively, constancy of carbonic acid concentration and constancy of bicarbonate concentration. Were either regulatory mechanism to fail the resulting alteration in reaction of the internal fluid environment would so modify cell permeability and ionic composition that vital enzyme systems could no longer function properly.

Nature of the Problem of the Renal Regulation of Acid-base Balance. The renal problem of stabilizing the concentration of bicarbonate† in the body fluids is a dual one. The first aspect of the problem is that of salvaging the large quantities of bicarbonate

† In reality the kidney stabilizes the concentration of base in the body fluids at a level some 25 to 27 mEq./L. above that of the sum of all the fixed non-volatile anions. This base is neutralized by the ever present carbonic acid (dissolved carbon dioxide) to form bicarbonate. Bicarbonate therefore serves as a measure of, or tags, that moiety of base which in large part accounts for the weakly alkaline properties of the body fluids, and which is readily available to neutralize any stronger acid produced in the body. In speaking of bicarbonate one must keep in mind that it is the base bound to this potentially volatile anion which is uniquely valuable to the organism, and that the anion itself is merely drawn from or returned to the metabolic stream of carbon dioxide as the occasion demands. Nevertheless, we shall adhere to common clinical parlance by referring to bicarbonate bound base as bicarbonate.

* From the Department of Physiology, State University of New York Medical Center at Syracuse University, Syracuse, N. Y.

which enter the renal tubules in the glomerular filtrate. Some 200 L. of plasma are filtered through the glomeruli each day. Since each liter of plasma normally contains 25 to 27 mEq. of bicarbonate, a total of 5,000 to 5,400 mEq., or about 1 pound when expressed as sodium bicarbonate, is delivered into the tubules every twenty-four hours. This is five times the amount of bicarbonate which the body actually contains. Obviously, bicarbonate must be filtered over and over again and reabsorbed each time by the renal tubules. The efficiency of the reabsorptive process is indicated by the fact that only 1 or 2 mEq. escape in the urine each day. However, if sodium bicarbonate is ingested or if the diet is such that it yields an alkaline ash residue, the excess bicarbonate is excreted in the urine.

The second aspect of the problem of renal stabilization of the concentration of bicarbonate derives from the fact that nonvolatile acids such as sulfuric and phosphoric are formed in considerable quantities in the metabolism of phospholipids and proteins. These acids, neutralized in the body by base contributed by bicarbonate, tend to deplete body stores. The depletion would proceed at such a rate as to exhaust the bicarbonate base reserves completely within a week were it not for two renal compensatory processes, namely, the excretion of titratable acid and the excretion of ammonia. In essence these two mechanisms reverse the process of neutralization, promote the elimination of acid anions minus the base which they bind in the blood, and restore that base to the body as bicarbonate.

In excreting titratable acid, neutral salts of weak buffer acids, which enter the renal tubules in the glomerular filtrate, are transformed into acid salts or even into free acids. The base is restored to the body as bicarbonate. In the example below, disodium phosphate is shown as reacting with carbonic acid to form sodium bicarbonate and monosodium phosphate.

$$Na_2HPO_4 + H_2CO_3 \rightarrow NaH_2PO_4 \text{ (excreted)} + NaHCO_3 \text{ (absorbed)}$$

The reaction is driven to the right by the reabsorption of sodium bicarbonate. The original filtrate which, like the plasma, has a slightly alkaline reaction (pH 7.4) is thereby converted into acid urine. If one collects the urine for twenty-four hours and titrates it from its initial acid reaction to the reaction of the blood, the amount of base which must be added is exactly equal to the amount of base which the tubules have recovered as bicarbonate in forming the acid urine. The capacity of the kidney to perform this operation is somewhat limited. Thus the tubules can salvage at most one of the two sodium ions bound by disodium phosphate. To salvage the second sodium ion would require the ability to produce urine of pH 2.5. Actually the most acid urine which the kidney can form is pH 4.5. For this reason it can excrete negligible quantities of strong acids such as sulfuric or hydrochloric as free titratable acid.

These strong acids must be eliminated fully neutralized but the kidney accomplishes this end without sacrifice of sodium ions by combining them with ammonium ions. In the example below, sodium sulfate, delivered into the tubules in the filtrate, is shown as reacting with ammonium bicarbonate to form sodium bicarbonate and ammonium sulfate.

$$Na_2SO_4 + 2NH_4HCO_3 \rightarrow (NH_4)_2SO_4 \text{ (excreted)} + 2NaHCO_3 \text{ (absorbed)}$$

Again the reaction is driven to the right by the reabsorption of sodium bicarbonate. If one collects the urine for twenty-four hours, its ammonia content, expressed in mEq., is exactly equal to the quantity of base which the tubules have recovered as bicarbonate in transforming salts of fixed base into ammonium salts. The sum of the titratable acid excreted and the ammonia eliminated is a measure of the total renal defense of the bicarbonate reserves. Under ordinary circumstances the kidney balances its acid-base budget by leaning somewhat more heavily on ammonia production than on titratable acid excretion. The two processes are,

however, intimately related functionally, a theme which we shall develop later.

Contributions of Titratable Acid and Ammonia Excretion to Regulation of Acid-base Balance. The contributions of these two processes to stabilization of the bicarbonate content and reaction of the body fluids are summarized in Table 1 compiled from a number of sources.[2–7] A normal individual on an average mixed diet excretes the equivalent of 300 to 500 cc. of 0.10 normal acid per day in combination with ammonia, and 100 to 300 cc. as titratable acid. In this manner he eliminates each day exactly that amount of acid which is produced in the oxidation of thioamino acids and phospholipids. His body stores of bicarbonate and the reaction of his body fluids are both maintained at optimum levels.

TABLE 1
MECHANISMS BY WHICH ACID IS EXCRETED WITHOUT LOSS OF FIXED BASE—SUMMARY OF QUANTITATIVE DATA ON MAN

	cc. 0.10 Normal Acid per Day	Ratio $\frac{NH_3}{Acid}$
Normal Man		
1. Excretion of acid combined with NH_3	300–500	1–2.5
2. Excretion of titratable acid	100–300	
Diabetic Acidosis		
1. Excretion of acid combined with NH_3	3,000–5,000	1–2.5
2. Excretion of titratable acid	760–5,000	
Nephritic Acidosis		
1. Excretion of acid combined with NH_3	5–150	0.2–1.5
2. Excretion of titratable acid	20–200	

The uncontrolled diabetic patient in severe ketosis produces much more metabolic acid than does the normal individual. Not only does he metabolize his own body proteins and lipids at a high rate, producing increased quantities of sulfuric and phosphoric acids, but he likewise produces large quantities of beta-hydroxybutyric and acetoacetic acid. The excessive drain on base stores in neutralizing this load of metabolic acid is reflected in a five- to tenfold increase in the rate of excretion of titratable acid and ammonia. Yet the renal compensation is never complete and moderate to severe acidosis develops; that is, the bicarbonate stores of the body are depleted and the blood and tissue fluids become more acid.

In nephritis the production of metabolic acid is within normal limits, or even reduced if the patient is fed a high carbohydrate, low protein and fat diet. However, the capacity of the kidneys to eliminate acid without loss of base is limited, and the more severe and long-standing the renal disease process the greater is the reduction in these renal capacities. In the terminal stages of Bright's disease total acid excretion may drop to 25 cc. of 0.10 normal acid per day, 5 cc. in combination with ammonia, and perhaps 20 cc. as titratable acid.

Properties of the Renal Mechanisms for Reabsorption and Excretion of Bicarbonate. The urine of the normal individual maintained on an average mixed diet is slightly acid, the reaction averaging pH 6.0. It contains negligible traces of bicarbonate. However, the ingestion of as little as 4 gm. of sodium bicarbonate causes the urine to become less acid[8] and much larger quantities of base may be readily eliminated in urine but slightly more alkaline than the blood plasma. In fact, the most alkaline urine which the kidney can elaborate is pH 8.0.

The relationships between blood plasma concentration and tubular reabsorption and excretion of bicarbonate in three normal adult subjects are illustrated in Figure 1.[9] The plasma level of bicarbonate in these experiments was varied over a range of 13 to 38 mEq./L. The low values were obtained by the ingestion of 10 to 20 gm. of the acidifying agent, ammonium chloride, on the day preceding an experiment. The plasma level was gradually elevated during the course of each experiment by the intravenous infusion of sodium bicarbonate. Measurements of filtration, absorption and excretion of bicarbonate were made at

intervals as the plasma level was raised from subnormal, through normal, to elevated levels.

In essence Figure 1 defines the renal threshold relationships for bicarbonate in normal man. At plasma concentrations ranging from 13 to 24 mEq./L., indicative of slight to moderately severe acidosis, all of the bicarbonate entering the glomerular filtrate is reabsorbed. None is allowed to escape in the urine. At plasma concentrations above 28 mEq./L. absorption is less complete. In fact, from each 100 cc. of filtrate 2.8 mEq. are absorbed; all in excess of this amount is excreted. The renal plasma threshold for bicarbonate therefore lies between 25 and 27 mEq./L.

The significant fact about this absorptive process is how nicely it is poised to stabilize the plasma bicarbonate concentration at 25 to 27 mEq./L. If body stores of bicarbonate are normal or reduced, the filtered bicarbonate is entirely absorbed. Such a state of affairs is the usual one, for we ordinarily do not have any excess of base at our disposal. However, if sodium bicarbonate is administered, the plasma concentration rises above the normal range. All present in the plasma, in excess of that amount which is optimal, is excreted. The urine becomes alkaline because of its increased content of bicarbonate-bound base.

The concept of a stable renal threshold for bicarbonate must be accepted with certain reservations: first, that the threshold is reduced by hyperventilation; and second, that it is increased in the presence of a low blood chloride. Forced breathing, induced voluntarily or reflexly in consequence of anoxia, lowers the bicarbonate concentration of the plasma yet leads to the excretion of bicarbonate and the formation of an alkaline urine.[2] Obviously, the capacity of the renal tubules to reabsorb bicarbonate must be reduced under these conditions but no evidence exists as to the nature or site of action of the stimulus which brings about this reduction. It is probable that changes in plasma pH may, in some way yet to be defined, exert a regulatory influence over bicarbonate reabsorption. It is well known that changes in plasma pH of considerable magnitude occur with hyperventilation.

In pernicious vomiting and in prolonged gastric drainage, or following repeated ingestion of large quantities of sodium bicarbonate, the concentration of chloride in the body fluids is reduced and that of bicarbonate is proportionately elevated. Despite alkalosis, which may be sufficiently severe to lead to tetany, the urine remains acid and contains essentially no bicarbonate.[10–20] So long as the blood chloride is depressed the kidney conserves bicarbonate. Only when the blood chloride is raised by the infusion of saline is the bicarbonate threshold restored to the normal range. It has been suggested that the kidney, failing to maintain normal proportions of chloride and bicarbonate in the body fluids, at least stabilizes total concentration. Furthermore, the increased absorption of bicarbonate which characterizes hypochloremic alkalosis has been assigned to the proximal tubule where, because of reduced plasma level, less than normal quantities of chloride are absorbed.[13]

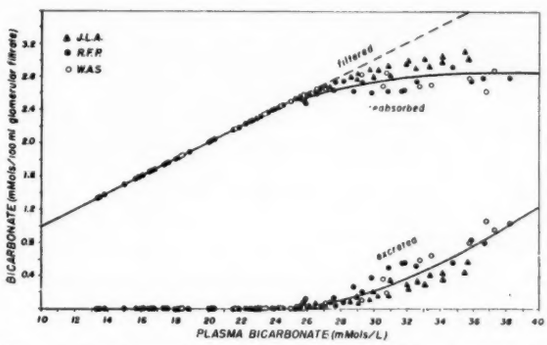


FIG. 1. Reabsorption and excretion of bicarbonate in normal man as a function of plasma level. (From Pitts et al.[9] Courtesy of *J. Clin. Investigation.*)

The acid-base relationships of the urines collected in the experiments described in Figure 1 are summarized in Figure 2. Urines containing negligible quantities of bicarbonate were more acid than pH 6.5; the minimum bicarbonate concentration was 0.00001 mEq./L. at pH 4.47. Urines containing appreciable quantities of bicarbonate were more alkaline than pH 7.0; the

maximum bicarbonate concentration observed in these experiments was 164 mEq. /L. at pH 7.80. According to Gamble[14] and Marshall[15] the carbon dioxide tension of the urine is equal to or greater than that of the blood. Because of the buffering action

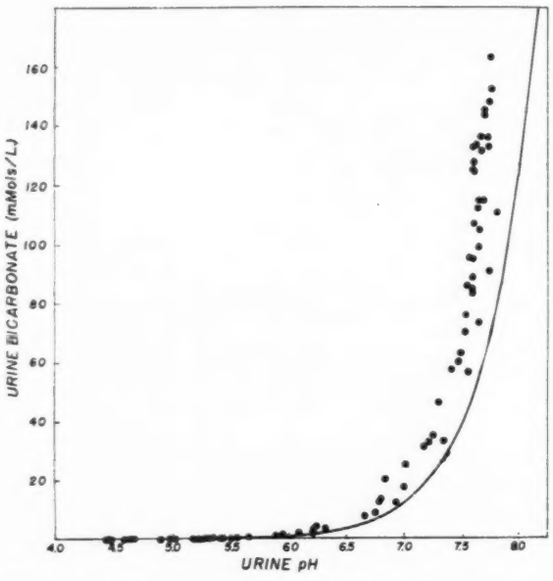


FIG. 2. Relationship between bicarbonate concentration and pH in eighty-two specimens of urine so collected as to prevent loss of carbon dioxide. (From Pitts et al.[9] Courtesy of *J. Clin. Investigation.*)

of the carbon dioxide (carbonic acid), they observed that as much as 220 mEq. of base bound as bicarbonate could be excreted in a liter of urine no more alkaline than pH 8.0. More recent studies have demonstrated that the carbon dioxide tensions of urines of high bicarbonate content are considerably greater than the plasma values, a fact which even further facilitates the elimination of base in urine of moderate alkalinity.[9,13] Our own experiments have shown that the tension of carbon dioxide may be three times that of the plasma from which it was formed. We shall return to this fact later in a discussion of the distal tubular mechanism of bicarbonate reabsorption. However, the extent to which it tempers the alkalinity of the urine is indicated in Figure 2. It is evident that all urine specimens which contained appreciable quantities of bicarbonate were less alkaline than would be expected were the carbon dioxide tension to be maintained constant at a normal venous plasma level of 50 mm. Hg, i.e., the circumstance described by the continuous line curve.

If there were no continued drain on bicarbonate-bound base to neutralize acids

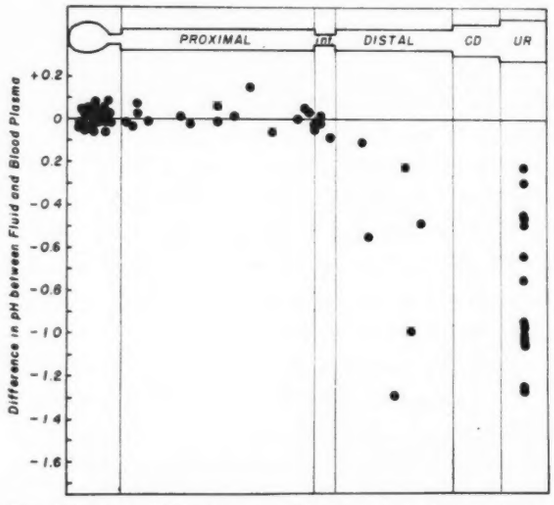


FIG. 3. Relationship between the reaction of the tubular urine and that of the plasma at various levels in the amphibian nephron. (From Montgomery and Pierce.[16] Courtesy of *Am. J. Physiol.*)

formed in fat and protein metabolism, or if one were to ingest each day an excess of sodium bicarbonate over and above that used in neutralizing these acids, the absorptive and excretory processes just described would serve to stabilize the plasma concentration of bicarbonate within the normal range. The fact that we need no continuous supply of exogenous base to maintain our bicarbonate reserves derives directly from an inherent renal capacity to regenerate our own endogenous supplies as needed. We do this by excreting acid anions in the form of free titratable acid and in combination with ammonia. The base with which these anions were neutralized in the body fluids is salvaged as bicarbonate.

Properties of the Renal Mechanism for Acidification of the Urine and Excretion of Titratable Acid. The studies of Montgomery and Pierce[16] on the amphibian kidney provide a morphologic basis for an understanding of the processes by which the renal tubules convert the slightly alkaline glomerular filtrate into acid urine. These investigators

withdrew minute quantities of fluid from the glomerulus and from the proximal, intermediate and distal segments of the nephron and determined the pH with a micro-quinhydrone electrode. Figure 3, taken from their work, compares the reaction of the fluid obtained at each level with that of the plasma from which it was formed. It is evident that within reasonable limits the pH of the glomerular filtrate and the tubular fluid obtained from the proximal and intermediate segments is identical with that of the plasma. In the distal tubules the urine becomes acid, the pH decreasing to values as low as those observed in urine obtained from the ureter and bladder. Acidification of the urine and excretion of titratable acid are thus functions of the distal segment of the renal tubule.*

There are, obviously, two general means by which the distal tubule could convert its slightly alkaline contents into acid urine: first, it might reabsorb the alkaline components of the buffer mixtures which enter the glomerular filtrate, leaving the acid residues to be discharged into the urine; second, it might add acid to the tubular contents. Four of the several possible permutations of these two basic hypotheses are described in Figure 4.

Only two buffer mixtures enter the glomerular filtrate in significant quantities, namely, monobasic and dibasic phosphate, and carbonic acid and bicarbonate. If, as shown in Figure 3 dibasic phosphate were preferentially reabsorbed, the excreted monobasic phosphate could be titrated as acid in the urine. This concept, which may reasonably be termed the *phosphate reabsorption theory*, has been rather widely accepted in the past. On the other hand, if bicarbonate were completely reabsorbed, and if the tubules were impermeable to carbonic acid, as claimed by Sendroy, Seelig and van Slyke[18] in their *bicarbonate reabsorption theory*, this acid would react with buffer salts to convert them quantitatively into free buffer acid.

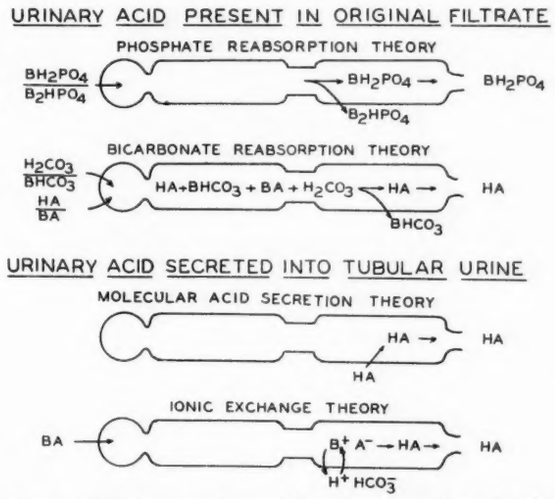


FIG. 4. Theories to account for the excretion of acid urine. (From Pitts et al.[21] Courtesy of *J. Clin. Investigation.*)

Similarly two hypothetic mechanisms depending on active tubular transfer have been proposed as explanations of urinary acidification. The *tubular secretion of molecular acid* has been invoked by Macallum and Campbell[19] to account for the conversion of alkaline buffer components into their acid forms. On the other hand, Homer Smith[20] has proposed an *ionic exchange mechanism* which would accomplish the same end. According to this latter view hydrogen ions, formed within the tubular cells by the dissociation of carbonic acid, are exchanged for ions of fixed base bound by buffer acids. To describe these latter two mechanisms as secretory is only partially justified, for reabsorption obviously plays a significant role in each.

It is possible to test certain of these theories experimentally in man, more specifically the first two, and to rule them out as incapable of explaining the known capacity of the kidney to excrete titratable acid.[21] The secretion of molecular acid with

* Whether this fact is equally true in the mammalian kidney has never been directly established. One might infer from the finding by Walker et al.[17] of chloride concentrations in proximal urine 30 per cent above the plasma values that the bicarbonate level must be nearly zero. If this were true the proximal urine would be appreciably more acid than the glomerular filtrate. There is in fact some indirect evidence that bicarbonate may be almost completely absorbed in the proximal segment when the plasma bicarbonate level is less than 20 mEq./L.

subsequent absorption of the salt formed cannot be excluded experimentally although there is indirect evidence that makes this occurrence unlikely.[22] Similarly the absorption of hydroxyl or carbonate ions,[23] with the consequent liberation of hydrogen ions

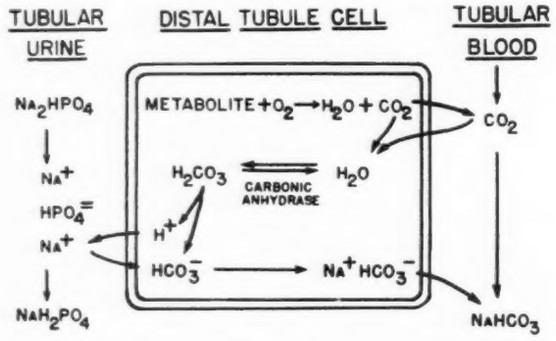


FIG. 5. Nature of the renal cellular mechanism for acidifying the urine. A single cell from the distal segment of the renal tubule is illustrated. (From Pitts and Alexander.[22] Courtesy of *Am. J. Physiol.*)

from water or bicarbonate, cannot be definitely excluded although the rapid rates of transfer and the infinitesimally low values to which the concentrations of these ions would have to be reduced makes it unlikely that appreciable formation of acid could result from such processes.[24] There remains as the most likely occurrence the process of ion exchange.

Our current view of the nature of the exchange mechanism is illustrated in Figure 5 by a diagram of a cell from that portion of the distal tubule which is concerned with acidification of the urine. Such a cell is exposed on one side to the tubular blood, on the other to the tubular urine. By virtue of its own metabolic activities, as well as its exposure to the renal capillary blood, a continuous supply of carbon dioxide is available to it. Because of its high content of carbonic anhydrase[25] the cell can rapidly transform this dissolved gas into carbonic acid. Hydrogen ions dissociated from carbonic acid are exchanged across the luminal border of the cell for ions of fixed base in the tubular urine. The base, along with an equivalent quantity of bicarbonate, is returned to the renal venous blood. The hydrogen ions, along with the anion residue, are excreted in the urine as titratable acid.

One of the complications observed in the early chemotherapeutic use of sulfonamides at high dose levels was the development of a moderately severe acidosis.[26] The unsubstituted sulfonamides, of which sulfanilamide is the only common example, are

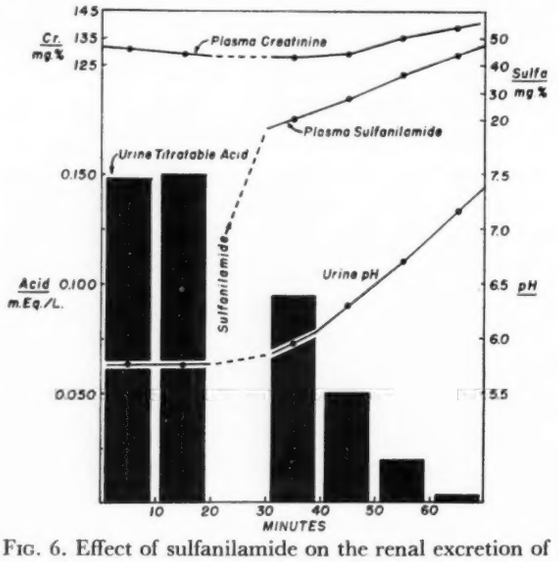


FIG. 6. Effect of sulfanilamide on the renal excretion of titratable acid in the acidotic dog. (Figure drawn from data of Pitts and Alexander.[22] Courtesy of *Am. J. Physiol.*)

known to block reversibly the enzymatic activity of carbonic anhydrase.[27,28] The effect of blocking carbonic anhydrase on the capacity of the kidney to excrete titratable acid is illustrated in Figure 6. In this experiment on a dog a moderately severe acidosis was induced by the administration of ammonium chloride. Titratable acid excretion was promoted by infusing creatinine to provide the kidney with a large excess of buffer upon which to operate. In the two control periods titratable acid, represented by the black columns, was excreted at a rate of 0.150 mEq./minute. A large dose of sulfanilamide was then given intravenously and the drug was infused at such a rate as to cause the plasma sulfanilamide level to rise from 20 to 45 mg. per cent in the course of the next forty minutes. Titratable acid excretion dropped nearly to zero and urine pH rose from 5.7 to 7.2.

Because sulfanilamide blocks the renal tubular replacement of sodium ions with

hydrogen ions, it promotes the urinary loss of base. That is, the base which would ordinarily be replaced by hydrogen ions and absorbed as bicarbonate is excreted in the urine in combination with buffer acids. Sulfanilamide, therefore, can and has been

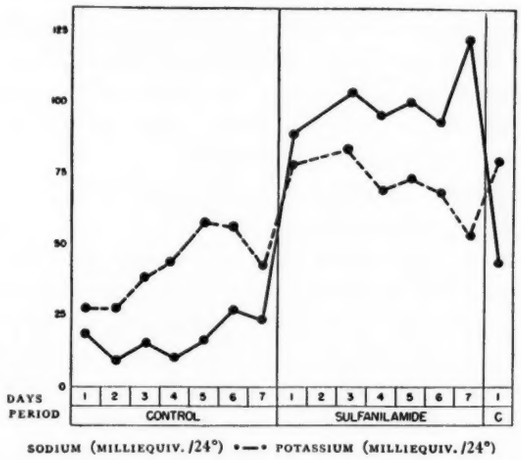


FIG. 7. Effect of sulfanilamide on the excretion of sodium and potassium by a patient in congestive heart failure. (From Schwartz.[29] Courtesy of *New England J. Med.*)

used as a diuretic in the treatment of the edema of congestive heart failure. During the seven-day control phase of an experiment by Schwartz,[29] summarized in Figure 7, the rate of sodium excretion of a patient in failure averaged 20 mEq. per day, or roughly 1 gm. when expressed as sodium chloride. During the next seven days 4 gm. of sulfanilamide were given each day. Sodium excretion increased fivefold, potassium excretion twofold. Loss of water in proportion to loss of ions resulted in a significant reduction in weight and in relief of dyspnea.

The schema of ionic exchange outlined in Figure 5 and the prominent role assigned to carbonic anhydrase in the aforementioned discussion undoubtedly oversimplify the situation, for the transfers outlined will not occur spontaneously. Energy must be cycled into the system in some way or other and the enzymatic reactions which provide the motive power, as well as the gearing by which that power is transferred to the cellular machinery, have been omitted. Crane, Davies and Longmuir,[30,31] studying the gastric mucosa, have proposed that electrical energy derived from the metabolic activity of the cell is used in an electrochemical process which results in a net separation of hydrogen and hydroxyl ions within the parietal cell. Carbonic an-

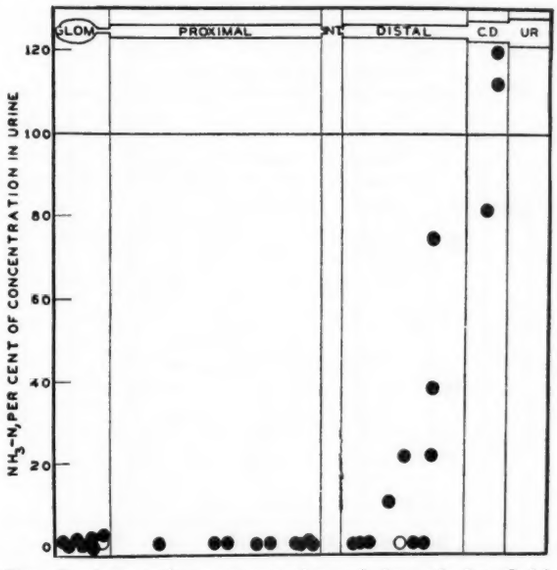


FIG. 8. Ammonia concentration of the tubular fluid at various levels in the amphibian kidney. (From Walker.[35] Courtesy of *Am. J. Physiol.*)

hydrase catalyzes the neutralization of the hydroxyl ions to bicarbonate ions. The hydrogen ions are transferred into the gastric juice, accompanied more or less passively by chloride ions. Patterson and Stetten[32] have extended these concepts by suggesting that the hydrogen ions are formed within the parietal cells by the reduction of pyridine nucleotide. Through the flavoprotein, cytochrome and cytochrome oxidase systems, electrons originating in this reduction are made available to reduce molecular oxygen to hydroxyl ions. Davies[33] predicts that all ionic secretory and absorptive mechanisms, including those of the kidney, will be found to have features in common.

Properties of the Renal Mechanism for the Excretion of Ammonia. To Nash and Benedict[34] belongs the credit for first demonstrating that the renal tubular cells form ammonia from some precursor in the arterial blood and secrete it in high concentration into the tubular urine. In Figure 8

are summarized experiments of Walker[35] which show that the secretion of ammonia, like the elaboration of acid urine, is a function of the distal tubule. Fluid drawn from the glomerulus and proximal tubule of the amphibian kidney contains only insignificant traces of ammonia. As fluid traverses the distal segment ammonia is added in increasing amounts.

TABLE II
GLUTAMINE AMIDE NITROGEN AS A SOURCE OF URINARY AMMONIA IN THE DOG*

Condition	Renal Plasma Flow (cc. per min.)	Ammonia-N Excreted (mg. per min.)	Glutamine-N Extracted from Plasma (mg. per min.)	Residual-N Extracted (Ammonia-N?) (mg. per min.)
Acidosis	245	0.562	0.330	0.232
	268	0.605	0.390	0.215
	262	0.615	0.410	0.205
Alkalosis	178	0.005	0.020	none
	200	0.004	0.020	none
	191	0.004	0.040	none

* From van Slyke et al.[37]

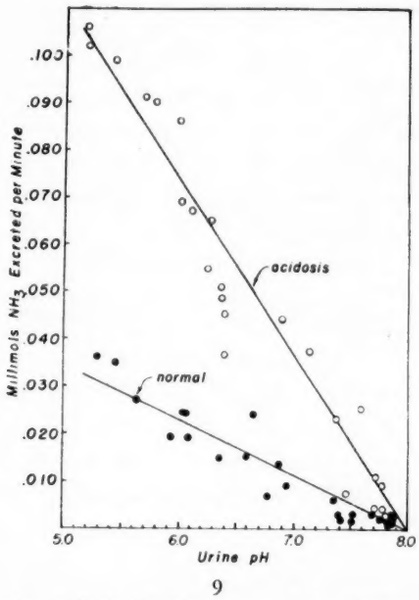
In the past there has been disagreement concerning the nature of the plasma precursors of ammonia although a majority have accepted urea as the probable source. Recently Archibald[36] demonstrated the presence of glutamine in the circulating blood plasma and of the enzyme glutaminase in the kidney. Following up these observations the group working in Dr. van Slyke's laboratory[37] showed in the acidotic dog that some two-thirds of the urinary ammonia is derived from the amide nitrogen of glutamine, and that none is derived from urea. The nature of the evidence in favor of this concept is illustrated by two experiments summarized in Table II, the first on a dog in acidosis excreting large quantities of ammonia, the second on a dog in alkalosis excreting essentially no ammonia. Blood was drawn from the renal artery and from the renal vein. The difference in glutamine concentration in the two samples multiplied by the quantity of blood plasma flowing through the kidney is a measure of the quantity of glutamine extracted by the tubular cells and presumably transformed into urinary ammonia. In the experiment in acidosis the quantity of glutamine nitrogen extracted from the blood could account for about two-thirds of the large quantity of ammonia nitrogen appearing in the urine. In the experiment in alkalosis essentially no ammonia was excreted and very little glutamine was removed from the blood flowing through the kidney.

Krebs[38] and others have suggested that urinary ammonia is derived in part from amino acids other than glutamine and have identified enzymes in the renal tubules capable of oxidatively deaminizing many of the naturally occurring amino acids. In Table II the third of the urinary ammonia not accounted for by the renal extraction of glutamine was presumably derived from amino acids. This concept has been strengthened by the demonstration that the intravenous infusion of a number of amino acids into acidotic dogs greatly enhances the excretion of ammonia.[39]

It is well established that the rate of ammonia excretion is proportional to the hydrogen ion concentration of the urine. The more acid the urine the greater is the rate of ammonia excretion. The more alkaline the urine the lower is its rate of excretion. The data presented in Figure 9 illustrate this fact well, and likewise the additional fact that urine acidity is not the sole factor governing ammonia excretion. All data in Figure 9 were obtained in experiments on one dog. In each experiment sodium bicarbonate was infused to raise the plasma level until it exceeded the renal threshold. As increasing quantities of bicarbonate appeared in the urine the pH of the urine increased. The series of data designated *normal* were obtained on infusing bicarbonate into an otherwise normal animal. In the series designated *acidosis* ammonium chloride had been administered at intervals over the preceding forty-eight hours to induce a moderately severe acidosis. It is apparent that some adaptation had

occurred during the two-day period of acidosis which greatly increased the animal's capacity to excrete ammonia at any given urine pH.

Two known facts indicate that the renal mechanism for ammonia excretion is closely related functionally to that for titratable acid excretion. First, ammonia excretion, like titratable acid excretion, mole for mole restores base to the body as bicarbonate. Second, the rate of ammonia excretion is inversely related to urine pH and hence must in some way be dependent on the mechanism of titratable acid excretion. Our views as to the nature of the interrelationships of the two mechanisms are outlined in Figure 10. If the tubular urine contained only salts of strong acids such as sodium chloride, the exchange of hydrogen ions for sodium ions could occur to a very limited extent, for the hydrochloric acid formed is highly dissociated. The high concentration of hydrogen ion attained in the urine would block appreciable transfer. The secretion of ammonia into the urine neutralizes this acid by binding the hydrogen ions as ammonium ions, thereby permitting the continued exchange of hydrogen ions for base. Each molecule of ammonia secreted into the urine binds one hydrogen ion and permits one sodium ion to be absorbed. Thus ammonia is exchanged for base mole for mole although the exchange is indirect.



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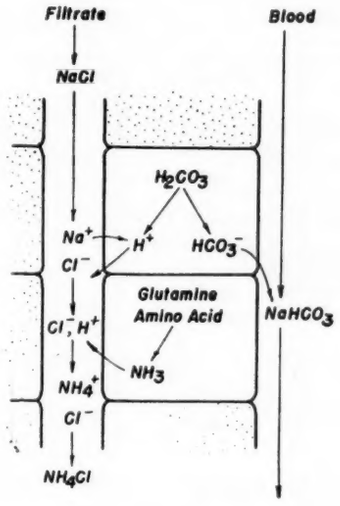

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FIG. 9. Relationship between rate of excretion of ammonia and urine reaction in a normal dog and in a dog rendered acidotic for forty-eight hours. (From Pitts.[24] Courtesy of *Federation Proc.*)

FIG. 10. Nature of the renal cellular mechanisms for secretion of ammonia. (From Pitts.[24] Courtesy of *Federation Proc.*)

The factor of urine pH plays its role by determining the rate of diffusion of free ammonia from its site of high concentration in the tubular cell to that of low concentration in the urine, where it exists not as free ammonia but as ammonium ion. Ammonia will diffuse rapidly into acid urine; it will diffuse scarcely or not at all into alkaline urine. As pointed out earlier the ammonia has its origin within the tubular cell in the oxidative deamination of amino acids and in the hydrolytic transformation of glutamine to glutamic acid.

The adaptation of the renal mechanism of ammonia excretion to acidosis can be partially related to the stimulation of the adrenal glands which occurs when body bicarbonate reserves are depleted. Figure 11 compares the renal responses of six normal rats and six adrenalectomized rats, maintained on a salt free diet, to the daily oral administration of 100 mg. of

ammonium chloride.[40] As shown in the upper left hand side of Figure 11 the normal rats took the procedure in stride whereas the adrenalectomized rats fell rapidly by the wayside. All of the adrenalectomized rats were dead by the fifth day. All of the normal rats were alive and

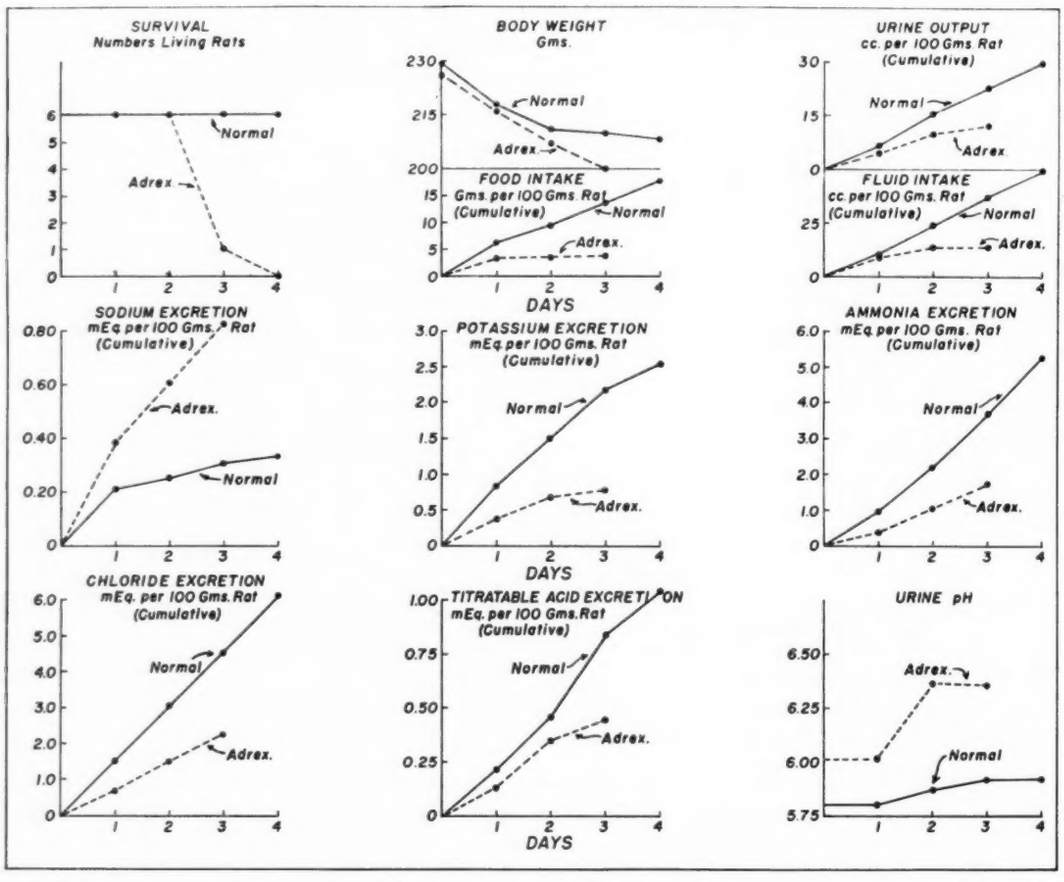


FIG. 11. Comparison of the response of normal and adrenalectomized rats maintained on a salt-free diet to the daily administration of 100 mg. of ammonium chloride. (From Sartorius and Pitts.[40])

healthy. Both groups lost weight for ammonium chloride is a moderately effective diuretic at this dose level.

The normal rats lost fair quantities of body sodium in the urine on the first day in neutralizing the acid load. Thereafter, they progressively stepped up their ammonia and titratable acid production and curtailed their sodium loss. Because they compensated so completely for the acid load it could have been kept up indefinitely. The showing of the adrenalectomized rats in comparison was a poor one. They continued to waste sodium in neutralizing their acid load. They failed to step up ammonia and titratable acid production adequately, went downhill rapidly and died with severe acidosis.

Other experiments indicate that this deficiency in the renal response of an adrenalectomized animal to acidosis can be corrected more or less completely with either desoxycorticosterone or adrenal cortical extract.[40] Furthermore, there is evidence of adrenal activation in acidosis, in that a decrease occurs in the content of ascorbic acid and cholesterol in the adrenal glands of a normal rat when a single large dose of ammonium chloride is administered. Nevertheless, a complete explanation of the adaptive response of the kidneys to acidosis is lacking, although a part of the adaptation is undoubtedly due to stimulation of tubular activity by the adrenal cortical hormone.

Interrelationships of Plasma Bicarbonate Level, Bicarbonate Absorption, and Ammonia and Titratable Acid Excretion. If the mechanisms for excretion of ammonia and titratable acid are to function properly to maintain an optimum concentration of bicarbonate in the body fluids, it is necessary that the rates at which they regenerate base by ion ex-

change should be directly governed by the state of the body base reserves. It is probable that the site at which this control is exerted is the distal segment of the renal tubule, and that the control is dependent on competition between bicarbonate and salts of fixed acids as donors of the base exchanged for hydrogen ions and ammonia.

Our concepts of the way in which the quantity of bicarbonate reaching the distal tubule governs the excretion of titratable acid are illustrated in Figure 12. When the bicarbonate concentration of the plasma and glomerular filtrate is low, essentially all is absorbed in the proximal segment and little or none reaches the distal segment where ion exchange occurs. The transformation of buffer salts to titratable acid proceeds unopposed and a highly acid urine is formed into which ammonia diffuses rapidly. In contrast, when the concentration of bicarbonate in plasma and filtrate is elevated, considerable quantities reach the distal segment. Bicarbonate and buffer salts compete as donors of base. In place of fixed titratable acid, carbonic acid is formed. The excess bicarbonate along with the unaltered buffer salts are eliminated in alkaline urine, into which little or no ammonia diffuses. The slow dehydration of carbonic acid to carbon dioxide accounts for the high gas tensions earlier pointed out as being characteristic of alkaline urine.

The relationship between the plasma concentration of bicarbonate and the activity of those renal mechanisms which defend the base reserves of the body is illustrated in Figure 13.

In this experiment the acid-base balance of an otherwise normal subject was changed from a state of moderately severe acidosis to one of mild alkalosis within a relatively short period of time by the infusion of sodium bicarbonate. Acidosis was induced by the ingestion of 20 gm. of ammonium chloride on the day preceding the experiment. Because the rate of buffer excretion is low in ammonium chloride acidosis, creatinine was infused throughout the course of the experiment at a constant rate to increase the elimination of titratable acid to levels sufficient for accurate evaluation. The rates of excretion of titratable acid and ammonia are plotted upward, that of bicarbonate downward from the base line through the middle of the chart to emphasize their opposite relations to base economy. Plasma bicarbonate was raised gradually by the infusion of sodium bicarbonate from 15.5 mEq./L. to over 29 mEq./L. as a linear function of time.

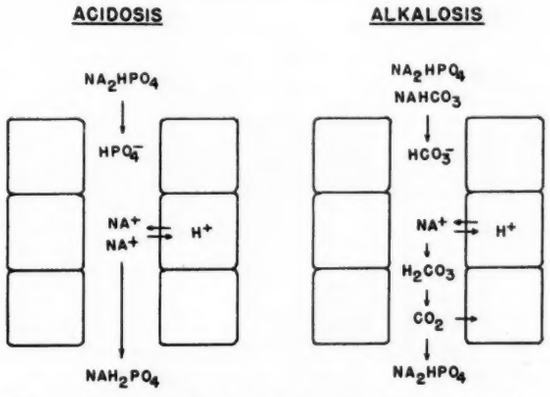


FIG. 12. Explanation of the inverse relationship between titratable acid excretion and the plasma level of bicarbonate. (From Pitts.[24] Courtesy of *Federation Proc.*)

Titratable acid and ammonia excretion were unaffected during the first sixty minutes of the experiment although plasma bicarbonate rose from 15.5 to 19 mEq./L. during this interval. In the fifth period at a plasma bicarbonate concentration of 20 millimols/L. the first perceptible reduction in acid and ammonia excretion occurred, a reduction which was progressive throughout the subsequent periods of the experiment. Not until the tenth period was any appreciable quantity of bicarbonate excreted. Thus from the fifth through the ninth periods the presentation of increased quantities of bicarbonate to the renal tubules and the reabsorption of that bicarbonate in some way diminished the excretion of titratable acid and ammonia by the tubules.

Our interpretation of this experiment may be appreciated by reference to the diagrams in Figure 12. The situation during periods 1 through 4 corresponds to that illustrated in the diagram designated *acidosis*. At plasma levels below 20 mEq./L. essentially all of the bicarbonate is absorbed in the proximal segment; little or none

reaches the distal segment; and base is exchanged for hydrogen ions and ammonia at maximal rates. When the plasma bicarbonate concentration exceeds 20 mEq./L., proximal absorption is incomplete and appreciable quantities of bicarbonate enter the distal segment. Bicarbonate-bound base competes with base bound by fixed acids in exchange for hydrogen ions. Less fixed titratable acid is formed, the urine is less acid in reaction, and in consequence less ammonia diffuses into the tubular urine. However, up to plasma concentrations of 25 to 26 mEq./L. the bicarbonate entering the distal tubule is completely absorbed by ion exchange. At plasma concentrations above 27 to 28 mEq./L. the absorption of bicarbonate in the distal tubule is incomplete, appreciable quantities appear in the urine and the reaction of the urine becomes definitely alkaline. Under these circumstances the excretion of titratable acid and ammonia approach zero; in fact, titratable alkalia is eliminated instead.

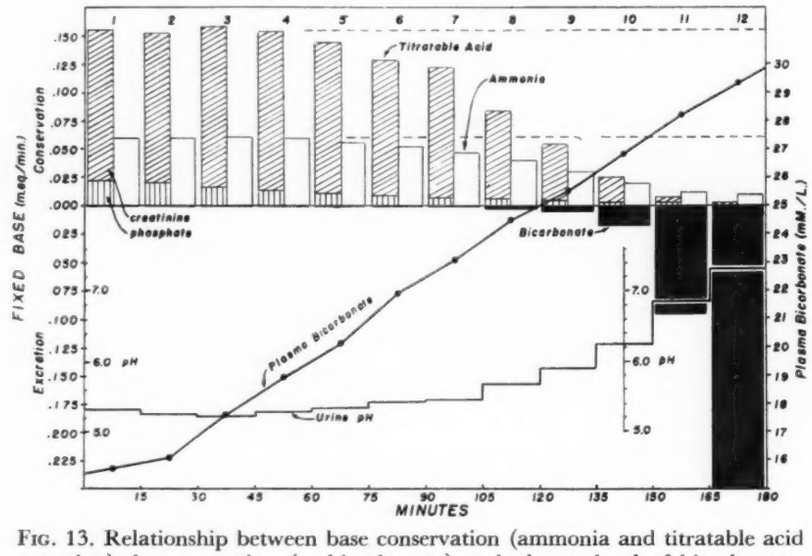


FIG. 13. Relationship between base conservation (ammonia and titratable acid excretion), base excretion (as bicarbonate) and plasma level of bicarbonate. (From Pitts et al.[9] Courtesy of *J. Clin. Investigation.*)

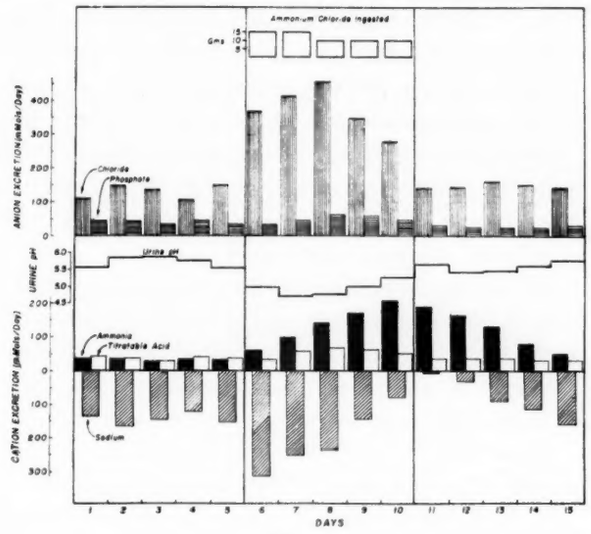
Mode of Operation of the Renal Defenses of Acid-base Balance in Health and Disease. The means by which the normal individual withstands the initial insult of a suddenly imposed acid load and the way in which his kidneys repair the damage to the acid-base structure of his body fluids are illustrated in Figure 14.[41]

For fifteen days the subject was maintained on a diet constant with respect to calories, protein and electrolytes. Successive twenty-four-hour urine samples were collected and analyzed for selected ionic constituents. The rates of excretion of these several constituents, expressed in milliequivalents per day, are plotted in block form. During the first five days, which constitute the control phase of the experiment, the net load of acid produced in the metabolism of the dietary protein and fat averaged 79 mEq. per day, 35 mEq. of which were excreted in combination with ammonia and 44 mEq. as free titratable acid. The subject was in electrolyte balance, excreting on an average 140 mEq. of sodium, 130 mEq. of chloride and 41 millimols of phosphate per day. In Figure 14 the excretion of sodium is plotted downward from that of ammonia and titratable acid to emphasize its opposite relation to base economy.

The acid load on the body was increased sharply during the second five-day period by the ingestion of 10 to 15 gm. of ammonium chloride per day. Fifteen gm. of ammonium chloride constitute a load equivalent to 2,900 cc. of 0.10 normal hydrochloric acid. It is evident that chloride excretion rose sharply on the first day of increased acid load. Urine pH dropped from a mean control level of 5.7 to 4.9; but since little hydrochloric acid can exist free in urine of

this reaction and since ammonia excretion was only moderately increased, the excess chloride was almost entirely neutralized by sodium* drawn from the buffer stores of the body. During the subsequent four days of acid ingestion this threat to the alkali reserve was met by the excretion of more titratable acid but, most disturbances had been corrected completely and conditions were back to normal.

An analogous experiment performed by Atchley, Loeb, Richards, Benedict and Driscoll[44] on a young severely diabetic patient is illustrated in Figure 15.



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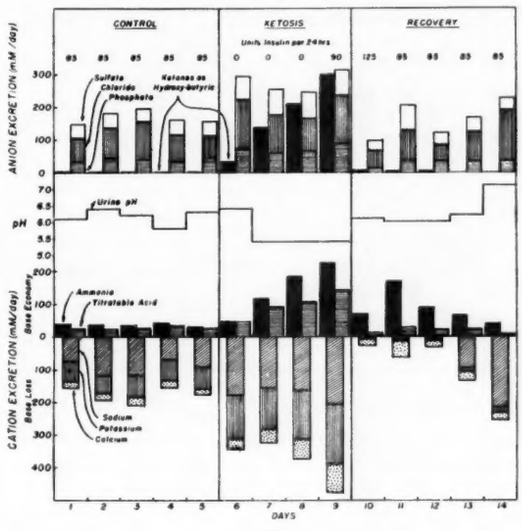

15

FIG. 14. Renal excretion of ions by a subject with normal renal function in ammonium chloride acidosis. (From Sartorius et al.[41] Courtesy of *J. Clin. Investigation.*)

FIG. 15. Renal excretion of ions by a diabetic subject in ketosis. (Figure drawn from the data of Atchley et al.[44] Courtesy of *J. Clin. Investigation.*)

significantly, also by the excretion of increased quantities of ammonia. As ammonia excretion increased base loss diminished in proportion until on the last day of acid ingestion a positive sodium balance was attained. Recovery had thus begun although the acid load was still great.

The processes of recovery and restoration of normal acid-base balance are well illustrated in the final five-day period of this experiment. On the first day of recovery essentially no sodium was excreted; all of that ingested in the diet was retained in the body. Anions were largely eliminated in combination with ammonia for the rate of excretion of ammonia was maintained near its peak level. Retention of sodium was evident for five days during which time the excess lost in the period of acidosis was restored to the body in full. On the sixth day of recovery, which is not shown in this chart, all

* Other experiments have demonstrated that potassium, calcium and magnesium neutralize a part of the urinary acid. Furthermore, in long-standing acidosis intracellular potassium stores may be seriously depleted by continued urinary loss.[42,43]

Their subject required 85 units of insulin per day for adequate control of his disease. Upon such a regimen excretion of ketone bodies was essentially zero and acid-base balance was attained during the control period by the elimination of 40 mEq. of acid in combination with ammonia and 30 mEq. in free titratable form, essentially the same quantities as in the previous normal subject. When insulin was withdrawn for four days, an increasingly severe ketosis developed, illustrated in the upper part of Figure 15, by a progressively increasing rate of excretion of beta-hydroxybutyric acid. This extra urinary acid was neutralized in part by the fixed bases, sodium, potassium and calcium, and in part by ammonia. A significant fraction was eliminated in free titratable form, for in urine of pH 5.3 roughly 30 per cent of beta-hydroxybutyric acid can exist as free acid. Despite increased excretion of titratable acid and ammonia, a severe acidosis developed and it was necessary to terminate the experiment by institution of insulin therapy. As in the normal subject the continued excretion of ammonia at

a high rate permitted the restoration of base reserves and the attainment of equilibrium by the fifth day.

This experiment and the previous one as well illustrate the capacity of the normal

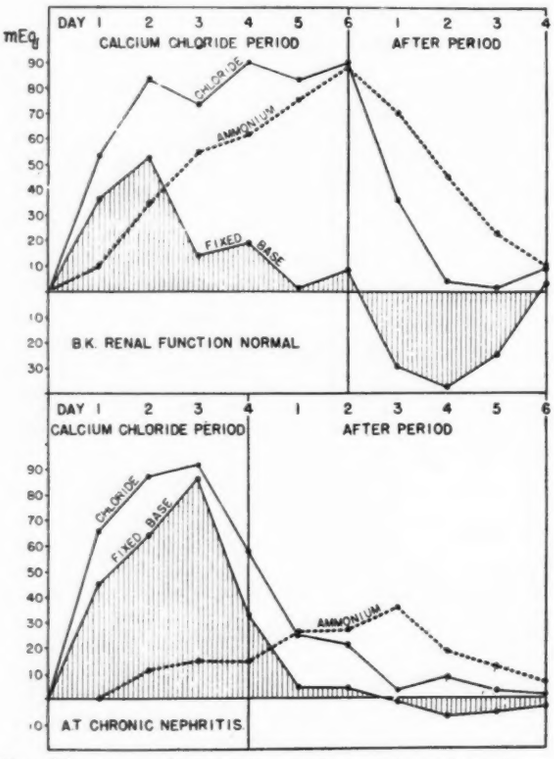

FIG. 16. Comparison of the renal excretion of ions by a subject with normal renal function and one with reduced renal function in calcium chloride acidosis. (From Gamble et al.[45] Courtesy of *J. Clin. Investigation.*)

kidney to correct disturbances of acid-base balance when provided with adequate quantities of fluid and sodium chloride. For such individuals alkali therapy of acidosis is unnecessary. The young diabetic, adequately hydrated with saline and glucose infusions and controlled with insulin, can correct a severe ketosis promptly without risking alkalosis. Such is not necessarily the case in the elderly diabetic who may exhibit relative or absolute renal insufficiency.

In Figure 16, taken from the work of Gamble, Blackfan and Hamilton[45] the response of a normal individual to a standard acid load is compared with that of a patient with chronic nephritis. Calcium chloride was administered as the acidifying agent. In each graph the urinary rates of excretion of chloride, fixed base and ammonia are plotted as increments over fore-period levels. It is evident in both the normal and nephritic subjects that early in the period of acid stress the excess urinary chloride was largely neutralized by fixed base. However, in the normal subject ammonia excretion increased promptly and by the sixth day was of sufficient magnitude to cover the chloride load. Loss of fixed base ceased, and in the succeeding recovery period depleted base reserves were restored promptly. In contrast, ammonia excretion in the nephritic subject increased but little. Loss of fixed base continued at a high rate and it was necessary to terminate the experiment on the fourth day because of the development of severe acidosis. Recovery was slow and inadequate.

SUMMARY

The kidney participates in the regulation of body neutrality by stabilizing the plasma concentration of bicarbonate-bound base at a level of 25 to 27 mEq./L. The respiratory system participates by stabilizing the plasma carbonic acid level at 1.25 to 1.35 mEq./L. Together the concentrations of these two components determine the reaction of the blood plasma and interstitial fluid which, under normal conditions, is maintained remarkably constant at pH 7.4.

The problem of the renal stabilization of the concentration of bicarbonate is a dual one, involving both salvage of the filtered bicarbonate and restoration to the body of the base utilized in neutralizing metabolic acids. In a quantitative sense, salvage of bicarbonate from the glomerular filtrate is the more significant for each day more than a pound of the sodium salt is absorbed by the renal tubules. The efficiency of the absorptive mechanisms is such that under normal conditions less than 0.10 per cent of that filtered is wasted in the urine. Nevertheless, following the ingestion of bicarbonate large quantities can be eliminated with only a slight increase in plasma level.

Somewhat more interesting from the

point of view of the physiologist and the clinician as well are the tubular mechanisms which substitute hydrogen or ammonium ions for sodium ions in the tubular urine. By virtue of these substitutions, metabolic acids may be excreted in free titratable form or in combination with ammonia, without sacrifice of the limited stores of body base. Both substitutions are carried out by the distal segments of the renal tubules, and certain enzymes, namely, carbonic anhydrase, glutaminase and a group of amino acid oxidases, have been assigned specific functions.

The acidosis of diabetic ketosis is primarily a consequence of flooding the renal tubules with such a load of metabolic acid that the exchange capacities of the tubules are overwhelmed. On the other hand, the acidosis of chronic renal disease is primarily a consequence of the inability of damaged renal tubules to exchange hydrogen ions and ammonia for base at a rate sufficient to compensate for a normal metabolic acid load. In either circumstance body base is drained away in neutralizing urinary acid. In chronic renal disease there is the additional factor of a reduced capacity to excrete phosphate and sulfate, both of which are filtered through the glomeruli and partially reabsorbed by the renal tubules. A reduction in the filtering bed accounts for a piling up of sulfate and phosphate in the body fluids with consequent displacement of bicarbonate.

Given a period of a few days in which to effect adjustments, normal renal tubules can increase their capacity to excrete acid more than tenfold. A part of this adjustment may be brought about by increased production of adrenal cortical hormones.

Acknowledgment: The author wishes to express his appreciation for the contributions of his colleagues, Drs. R. S. Alexander, W. D. Lotspeich, W. A. Schiess, J. L. Ayer, O. W. Sartorius and J. C. Roemmelt to the work described herein which originated in this laboratory, and to the United States Public Health Service and the John and Mary R. Markle Foundation for financial support.

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Conference on Therapy

Treatment of Neurosyphilis

THESE are stenographic reports of conferences by the members of the Department of Pharmacology and of Medicine of Cornell University Medical College and New York Hospital, with collaboration of other departments and institutions. The questions and discussions involve participation by members of the staff of the college and hospital, students and visitors. A selected group of these conferences is published in an annual volume, Cornell Conferences on Therapy, by the Macmillan Company.

DR. WALSH MCDERMOTT: The topic of the conference this afternoon is one of the two most important complications of syphilis. Up to about ten or fifteen years ago it was customary to believe that almost every kind of trouble was in store for the patient with syphilis. In more recent years the problems of the patient with syphilis have taken on a different aspect. Syphilitic infection during the early stages is now looked upon as a benign disease which causes no serious damage in most individuals. In the main, only two factors now stand out as serious problems in the infected person; they are syphilis of the aorta and syphilis of the nervous system. Of the two, neurosyphilis is by far the more important. The discussion of this subject will be opened by Dr. Webster.

DR. BRUCE WEBSTER: Since the treatment of syphilis of the central nervous system varies with the type of the disease, it is perhaps well to consider for a moment the matter of classification. By far the most frequent is the asymptomatic variety. It is recognized by the presence of a "positive" spinal fluid. The symptomatic variety falls into two general categories, namely, the inflammatory group and the degenerative group. In the inflammatory group, acute syphilitic meningitis and meningovascular syphilis are the most important. The degenerative group includes tabes, paresis and primary optic atrophy. It is well to bear in mind that, due to the diffuse nature of the infection, combinations of the aforementioned categories may occur in any one individual.

I should like to re-emphasize what has often been stated, namely, that prevention by the adequate treatment of early syphilis is the best form of therapy for syphilis of the central nervous system. If early syphilis were adequately cared for, this would be eradicated.

The evaluation of therapy for syphilis of the central nervous system must take into account many factors. First, there is the type of disease. Other factors are the race, sex and age of the patient, the duration of the syphilitic infection, the type and amount of treatment previously received, and the type of spinal fluid change.

At the present time considerable controversy exists among authorities on this disease as to whether therapy should be evaluated on clinical grounds or on the basis of changes in the spinal fluid. It is important to remember that in the degenerative types of the disease, antisyphilitic therapy cannot be expected to restore destroyed nerve cells. For this reason Dattner and Thomas have stressed the importance of evaluation of therapy on the basis of changes in the spinal fluid. Such an evaluation must take into account the following: (1) the cell count; (2) quantitative determination of protein; (3) quantitative titration of complement fixation; (4) colloidal gold test. If the cell count and spinal fluid protein are above normal, they consider the spinal fluid as "active." In other words, the advocates of this view believe that if the spinal fluid cell count and protein content become and remain normal as a result of treatment, even though the quanti-

tative Wassermann and colloidal gold curve remain fixed, the process in the nervous system, regardless of its type, has been rendered "inactive" and non-progressive whether or not the patient has achieved any degree of clinical improvement. Under such circumstances they consider further treatment useless. Conversely, Dattner and Thomas believe that if the cell count and/or protein content of the spinal fluid do not become normal after treatment or if following a period of return to normal the values again become abnormal, the patient should be re-treated.

Other authorities are not in agreement with the concept of what is referred to as "treating the spinal fluid and not the patient." Many patients show inactive spinal fluids before treatment but give evidence of clinical progression of the disease. The patient or his family is much more interested in relief of the symptoms and the maintenance of health. Unfortunately, this does not always coincide with inactivity of the spinal fluid. Both the spinal fluid changes and the clinical status must be considered in evaluating therapy.

Time will not allow a detailed discussion of the older methods of treatment of syphilis of the central nervous system. The results of the so-called intensified routine chemotherapy with arsenic and bismuth, either alone or combined with fever, are listed in all standard textbooks and may serve as a basis of comparison in the evaluation of the newer forms of therapy. Although induced fever has been used for twenty years, authorities are not yet in complete accord as to the value of this measure, applied in the form of malaria or artificial fever, in the treatment of syphilis of the central nervous system syphilis.

After the report by Mahoney, Arnold and Harris in 1943, pointing to the effectiveness of penicillin in early syphilis, clinical trials with penicillin in the central nervous system forms of the disease were made. In a cooperative study sponsored by the National Institute of Health considerable information has been accumulated in approximately 5,000 cases of syphilis of the central nervous system in which the treatment was either penicillin alone or penicillin and fever therapy. The results have recently been summarized in a report to the Council on Pharmacy and Chemistry of the American Medical Association by the Syphilis Study Section of the National Institute of Health.* Crystalline penicillin G is the product of choice in the treatment of syphilis in man. It is relatively non-toxic. The only reactions, other than those of the Jarisch-Herxheimer type, are allergic in nature and are mild. This fact alone makes penicillin a highly desirable agent in the treatment of neurosyphilis.

The dosage of penicillin used has ranged from 4 to 10 million units given over periods of from seven to twenty-one days. In the New York Hospital we have used a total dosage of 4.2 million units given over a period of fourteen days. This was formerly administered in about 25,000-unit doses at intervals of two hours but is now administered as a single daily injection of 300,000 units. When fever was used at all, it was in the form of malaria or artificial fever induced by the diatherm.

Two schools of thought appear to be emerging: One group advocates the use of penicillin alone in all forms of neurosyphilis. The patient is re-treated if after the first course signs of relapse appear, either clinical or in the spinal fluid. The proponents of this plan maintain that the results with penicillin alone are better than those with the older methods of chemotherapy and are at least as good as chemotherapy combined with malaria; and further, that penicillin possesses the advantage of complete safety from serious reactions. The other groups of investigators advocate the use of penicillin alone in the asymptomatic type, and also in the inflammatory type of symptomatic neurosyphilis, but urge concurrent fever and penicillin in the degenerative types, such as tabes, paresis and optic atrophy. Fever therapy may consist of the standard ten to twelve paroxysms above 39.3 degrees

* *J. A. M. A.*, 136: 877, 1948.

or may be the milder course of six to eight paroxysms as advocated by Solomon.

There appears to be general agreement that penicillin alone exerts a profoundly favorable effect in asymptomatic syphilis of the central nervous system. The cell count of the spinal fluid returns to normal in from two to six months after treatment. The protein is the next to attain the normal level. The Wassermann reaction and the colloidal gold tests show return toward normal more slowly. In the inflammatory types of neurosyphilis, i.e., acute syphilitic meningitis and meningovascular syphilis, the results of penicillin therapy equal or exceed those of chemotherapy.

In paresis, tabes dorsalis and primary optic atrophy, the results are perhaps more controversial. Nerve tissue already destroyed cannot be restored. Some of the disagreement among observers may depend on differences in the type of patients. Many of the large syphilis clinics deal, in the case of paretics, with patients who are ambulatory and who do not have to be confined or restrained. The results with penicillin therapy alone in these have been, on the whole, satisfactory. On the other hand, Solomon, dealing with psychotics in more advanced stages of the disease, may achieve better results with therapy in which both fever and penicillin are used. Again, the basis for some of the controversy may be the fact that the patients in the various clinics have not been entirely comparable.

Penicillin certainly extends the life expectancy of paretics. Many have been able to return to work. From the present evidence, approximately 50 per cent show definite improvement. In the others the condition remains unchanged or deteriorates. From 6 to 10 per cent succumb despite treatment.

The recent report to the Council on Pharmacy and Chemistry indicates that the value of adding malaria to treatment with penicillin is inconclusive. As I have stated, the difference in results with and without malaria may depend upon the stage of the disease at which therapy is instituted.

Penicillin produces striking relief of some of the clinical symptoms in many tabetics. Data are not yet available as to whether it will permanently arrest the tabetic degenerative processes. However, it is generally agreed that penicillin is more effective in tabes dorsalis than arsenic and bismuth. Whether or not malaria will augment the results is still uncertain.

Just as the results of the treatment of primary optic atrophy with chemotherapy and malaria were far from satisfactory, so is the treatment with penicillin and malaria. However, the latter appears to offer the best form of therapy available at the present time.

The policy we have adopted in the Syphilis Clinic of the New York Hospital offers a "middle of the road" plan. We prefer to use, as a rule, 4.2 million units of penicillin in fourteen days as the first course of treatment of all forms of syphilis of the central nervous system, except primary optic atrophy. If the clinical course of the disease or the spinal fluid change progresses unfavorably after this therapy, it is our policy to give another course of treatment with penicillin alone or penicillin combined with malaria, depending on the urgency of the situation and the patient's general condition. In all cases of primary optic atrophy we have used penicillin combined with fever. Follow-up at frequent intervals with combined clinical, psychometric and spinal fluid examinations is essential in order to evaluate the results of therapy in syphilis of the central nervous system.

In conclusion, it would appear that penicillin alone, in adequate dosage, given over a sufficiently long period of time, is a safe and effective form of treatment in most types of syphilis of the central nervous system. Further evidence is necessary to determine whether or not the addition of fever therapy enhances the effectiveness of penicillin therapy in the forms of the disease which are resistant to treatment with penicillin alone.

DR. MCDERMOTT: The conference is now open to questions. Dr. Wolff, would you care to comment?

DR. HAROLD G. WOLFF: The symptoms which are most troublesome to the clinician are those which occur late in the course of tabes when the patient is referred to as a case of "burned-out" tabes. I refer particularly to the tabetic crisis and root pains, which ordinarily occur in patients whose spinal fluid and blood are normal, and also cases of optic atrophy. Is there anything to be said about the management of these difficult conditions? I understand that some cases of optic atrophy are substantially benefitted by the use of malaria. I am also told that the process is sometimes arrested and that if it involves only one eye, the other eye fails to become involved. What can be said about the effects of malaria in root pains of the tabetic crisis?

DR. MCDERMOTT: Dr. Webster, would you care to speak on these points?

DR. WEBSTER: At a recent symposium on syphilis the various forms of therapy for primary optic atrophy were extensively discussed. I find myself on the fence regarding the benefit of any therapy for optic atrophy. There has always been the hope that the disease can be arrested, but the situation is now becoming one in which those who have had a great deal of experience are growing more and more pessimistic about the results of treatment of primary optic atrophy due to syphilis. This was reflected in the recent summary on this subject by the Syphilis Study Section which concluded that there was not enough evidence for a comparison of the results of penicillin and malaria with those of other chemotherapy and malaria. Our own experience is also inconclusive but it is by reason of this very lack of conviction that we continue to use malaria in primary optic atrophy.

DR. MCDERMOTT: Does not the evidence show that malaria without chemotherapy is of no use at all?

DR. WEBSTER: Yes, but I am pessimistic as to the effect of any form of therapy of this condition, although here and there something seems to happen to make us suspect that the treatment might have arrested the disease.

DR. MCDERMOTT: Did I understand you to say that the course of a tabetic's pains is altered by penicillin?

DR. WEBSTER: I might begin the answer by stating that it was our belief that thiamine chloride, which we used for a number of years, provided distinct relief of symptoms. There are, however, many factors which might enter into such relief, namely, changes in climate, suggestion and others. In the presence of an active spinal fluid we believe that penicillin therapy benefits the patient beyond a question of doubt. In the so-called "burned-out" individuals with negative spinal fluid there is no satisfactory evidence of benefit.

DR. MCDERMOTT: The situation in tabes is a good example of the fact that the diagnostic and therapeutic aspects in syphilis have advanced far more than our understanding of the disease. It is difficult to understand how an antimicrobial agent could possibly affect the pains of tabes, because such an agent could exert an influence only on the microbes. It is difficult to conceive of an infection lasting twenty years, progressing in no other way than to produce bouts of severe pain at long intervals. Yet that is exactly what seems to happen. I must admit that it is extremely difficult to arrive at a conviction regarding the value of a new agent for the relief of pain in a patient with tabes but I may express my belief that I have never been convinced that any of the agents previously used, namely, malaria, metals or vitamins, significantly altered the pain. I am inclined to believe that penicillin has somewhat altered the situation and that this agent does influence these pains, but it is my impression that the effect is not very substantial. I believe you have in your series patients in whom gastric crises while receiving penicillin have developed.

DR. WOLFF: What are the current views concerning the role of malaria in these

various types of syphilis? Is its effect in any way different from that of raising the temperature by some hot-box method?

DR. MCDERMOTT: That also is an unsettled question. A special immunity factor is a possibility in the case of malaria. However, fever induced by mechanical means produces results difficult to distinguish from those of fever induced by malaria. It is extremely likely that there is no difference in the mode of action.

DR. WEBSTER: It might be interesting to mention at this point that some benefit is derived from shock therapy and penicillin.

DR. WOLFF: In what group?

DR. WEBSTER: In paretics.

DR. MCDERMOTT: Are there other questions?

DR. MCKEEN CATTELL: Have you thrown the arsenicals out entirely in the treatment of neurosyphilis?

DR. WEBSTER: I would say that there is no question at all of the desirability of discarding tryparsamide. There is so much danger in this drug. Penicillin accomplishes more in a much shorter period of time. I would go further and state that the same applies to other forms of arsenic and bismuth. Penicillin produces little or no toxic effects and is just as effective therapeutically as arsenic and bismuth. Furthermore, there is the important factor of the markedly shortened period of treatment in the case of penicillin. I believe there is now general agreement that it is possible to accomplish as much in all forms of neurosyphilis with the use of penicillin as with arsenic and bismuth.

DR. CATTELL: Do you not use arsenic in this hospital?

DR. WEBSTER: No.

DR. MCDERMOTT: Those who still hold to arsenic and bismuth are, I believe, ultraconservative. I do not think there is the slightest doubt that penicillin is more effective than any other chemotherapy as a therapeutic agent for syphilis of the central nervous system. It was never possible to produce the profound and extensive cerebrospinal changes with the agents used prior to penicillin.

DR. RALPH R. TOMPSETT: I wonder if Dr. Webster would enlarge on his statement about the Herxheimer reactions? Was he referring to the Herxheimer reactions in neurosyphilis and, if so, are they dangerous? He also referred to allergic reactions in penicillin therapy. Would he say more about these?

DR. WEBSTER: The subject of allergic reactions is a fairly large one. I was referring simply to the ordinary hypersensitivity reactions which are encountered in some individuals following the administration of penicillin. There has been some reference in the literature to Herxheimer reactions in neurosyphilis, especially in paretics and, to a lesser extent, in tabetics. A few observers have noted that the lightning pains in tabes are increased during the period of penicillin therapy. Others have reported that the degree of psychosis is increased in paretics. These observations have not been sufficiently confirmed and I do not believe that enough information concerning them is available to decide whether they are the result of therapy or merely coincidental. Drs. Moore and Farmer reported a few cases in which a rise in temperature followed the administration of penicillin to patients with neurosyphilis but there were no untoward effects and these few may have been coincidental.

DR. MCDERMOTT: Are you afraid of a Herxheimer reaction in neurosyphilis, Dr. Webster?

DR. WEBSTER: I am not. We have had no such reactions here. It is only fair to state that there are some who hold a contrary view.

DR. MCDERMOTT: I am inclined to agree with you. Spirochetes are necessary to produce the reaction, which reflects the killing of spirochetes. The reaction is transient. I should like to stress the desirability of going ahead with the treatment. It should not be interrupted because of the reaction. The reports on Herxheimer reactions in tabes are in need of careful scrutiny since a true

Herxheimer reaction would involve the assumption that there are spirochetes in tabes.

DR. WOLFF: Would you say something about the public health aspect of the new treatment? Is there any evidence that the intensive preoccupation with treatment of very early syphilis is reducing the amount of neurosyphilis?

DR. MCDERMOTT: It is too early for a conclusive answer to that question. The amount of early neurosyphilis occurring as a relapse of infectious syphilis treated with penicillin, or treated with other rapid methods for that matter, is very small indeed. If the patients had only one syphilitic infection and if that were treated with penicillin, I have little doubt that the incidence of neurosyphilis would decline. However, there is the point that some of these patients have three, four or five infections of syphilis, and how that would affect the incidence of late neurosyphilis only time can tell.

DR. WEBSTER: Don't you think we shall have to wait ten or fifteen years, a time sufficiently long to give these patients an opportunity to develop neurosyphilis, before the effect of the treatment on the incidence of neurosyphilis can be properly judged?

DR. MCDERMOTT: Perhaps it does not have to take so long. I believe that in the patient who is free from neurosyphilis two or three years after the initial syphilis, and who does not acquire the disease again, neurosyphilis will not develop. I think the patient can be assured early in the course that the chance of having neurosyphilis develop is negligible, provided these conditions prevail, namely, effective treatment of early syphilis; absence of reinfection and absence of neurosyphilis after a lapse of two or three years following the initial attack. That may not apply to the development of tabes in all cases of syphilis. We simply do not understand the conditions which apply to tabes.

Dr. Webster, would you tell us something about your plan for follow-up? How long does one pursue it and what criteria are used?

DR. WEBSTER: I might describe the routine we use here. We try to see all patients with neurosyphilis treated with penicillin at intervals of a month after their discharge from the hospital. We endeavor to examine the spinal fluid at intervals of three months in the first year. If the spinal fluid becomes negative and remains so for a year, the intervals between examinations are prolonged. On the other hand, if the abnormal findings in the spinal fluid remain unchanged, the follow-up is made more intensive. All patients with syphilis of the central nervous system should remain under observation for life.

DR. WOLFF: I wonder if we could have some more discussion of the laboratory tests used as guides to improvement after treatment with penicillin alone, or by combined treatment.

DR. MCDERMOTT: The evidence is fairly good that the abnormal findings in the spinal fluid, namely, increase in cell count, increase in total protein and the abnormal protein reflected in the gold curve, disappear with approximately the same speed under treatment with either penicillin or malaria. The same is true of the Wassermann test but modifications of the complement fixation tests have greatly increased their sensitivity. For these to become totally negative would frequently take as long as four or five years.

DR. WOLFF: Do you refer to both blood and spinal fluid?

DR. MCDERMOTT: I was speaking only of the spinal fluid. It is easier to reverse serologic tests of the blood than of the spinal fluid. In a long-treated neurosyphilitic patient it is not uncommon to find the blood serology negative but the spinal fluid quite "active."

DR. WEBSTER: Do you believe that the type of syphilis would have a bearing on this point? Do you think that the inflammatory types, such as acute syphilitic meningitis and possibly meningovascular syphilis, show improvement in cells and

protein of the spinal fluid more rapidly after penicillin than after malaria?

DR. MCDERMOTT: I am glad you brought up these points. It is possible to compare the two agents only in those types in which one or the other is used. Syphilitic meningitis has never been treated with malaria.

DR. WOLFF: Could you indicate the general course of the changes in the Wassermann test during the first few months of vigorous treatment and also the changes that one might expect in the ensuing four or five years?

DR. MCDERMOTT: If one starts with a six-tube titer, at the end of one year after completion of treatment it may go down to four or a little less. At the end of two to four years only a very low titer may remain. In such a case, it would be wise to have the tests made by the same laboratory under the same conditions. A titer which has become very low may be found positive by one laboratory and negative by another.

DR. WOLFF: I take it then that after the complement fixation test has fallen to a 2 plus you might allow the patient to carry that the rest of his life.

DR. MCDERMOTT: If the situation in syphilis is compared with that of other infectious diseases, the persistence of positive serologic reactions cannot be regarded as harmful. When the syphilologist aims at absolute disappearance of positive serologic reactions, he is aiming at a result impossible to achieve in other infections. Sociologic and emotional factors associated with the presence of positive reactions provide a strong pressure behind the aim for serologic reactions. I am sure that as many people are injured by the knowledge that they have syphilis, even of an inconsequential nature, as are harmed by the organic changes of syphilis.

DR. WEBSTER: Don't you think that the longer the patient has had syphilis of the central nervous system the more difficult it may be to reverse positive serologic reactions?

DR. MCDERMOTT: I do not know whether it is a general rule. Your reference to the rapid reversal in the case of acute syphilitic meningitis supports the view. However, whether a difference would be in evidence in a comparison between, for example, a disease with a duration of five years and one of ten years, I cannot be sure.

DR. WEBSTER: I have the impression that the reversal of positive serologic reactions is more readily achieved in early than in long-standing cases.

DR. MCDERMOTT: That seems reasonable.

DR. WEBSTER: It is important to bear in mind that in some patients the positive reaction of the spinal fluid cannot be reversed regardless of the amount of treatment. As Dr. McDermott has pointed out, after a vigorous course of therapy the syphilitic process is probably arrested and nothing is to be gained by continuing treatment for the rest of the patient's life.

DR. WOLFF: May I ask another question? Is there any new information regarding spontaneous reversal of syphilis of the central nervous system?

DR. MCDERMOTT: As far as I know there is none. For information concerning a spontaneous cure of syphilis we fall back on the report of Luusgaard about twenty years ago. Although that study fails to meet entirely satisfactory scientific standards, it is a notable contribution. It calls attention to the fact that many patients with syphilis are not seriously affected by it.

VISITOR: Is there any information available on the course of syphilis acquired simultaneously with gonorrhea? I refer to patients who acquire both diseases in the same exposure, who receive a few "shots" of penicillin, and subsequently turn up with positive spinal fluid or blood serologic reactions.

DR. MCDERMOTT: I have forgotten the exact incidence of this combination but I believe it is in the neighborhood of 2 per cent. Would you know exactly, Dr. Heimoff?

DR. LEONARD L. HEIMOFF: It is from 2 to 4 per cent.

DR. MCDERMOTT: Among men in the Army with acute gonorrhea who received penicillin therapy for the gonorrhea 2 to 4 per cent were subsequently found to have

infectious syphilis, presumably acquired from the same exposure. Those represent partially treated syphilitic infections in which the host-parasite relationship may have been upset by the penicillin.

DR. HEIMOFF: The only difference we observed in these mixed infections was a delay in the incubation period of syphilis. It took about a week longer for the chancre to appear; it appeared in a month rather than in two or three weeks. There is also the fact that in most of them the chancre did not appear at all, but a positive serology developed in four to six months rather than in the more usual period of three months. We had to follow these cases serologically for four months before it was safe to discharge them from observation.

DR. MCDERMOTT: In other words, a man treated for gonorrhea was also observed for a period of four months as a syphilitic suspect, is that so?

DR. HEIMOFF: Yes.

DR. MCDERMOTT: Was that only the case when the patient had received penicillin?

DR. HEIMOFF: Yes.

DR. MCDERMOTT: Dr. Wolff, have you any notion as to how the pains of tabes are brought about? I vaguely recall a patient with some type of tumor, whom you showed us at Grand Rounds about a year ago, in whom you postulated vascular reflexes as an explanation of paroxysms of pain.

DR. WOLFF: Your memory of the case is correct. It was my notion that the tumor, by irritating the sensory root, reflexly produced vasoconstriction in the sensory root. The tumor was pressing more or less continually but the pain was paroxysmal.

DR. MCDERMOTT: Do you assume a similar mechanism as the cause of the pain in tabes?

DR. WOLFF: That explanation has seemed attractive to me since the dorsal root ganglion has such a limited capillary supply, about as little blood supply as in ordinary white matter. There is little margin of safety and any minor vascular disturbance might readily give rise to spontaneous discharge of a painful nature. What, in turn, initiates the vascular discharges, we do not know. It may be some minor change in the neighborhood, such as slight edema, which may have nothing to do with the syphilis itself. Yet the faint restriction of the blood supply may give rise to the painful reaction.

DR. MCDERMOTT: That is a very attractive hypothesis to explain such paroxysms of pain in a state of a disease which is more or less constant for years.

DR. WEBSTER: I think climatologic factors could explain such paroxysms.

DR. WOLFF: I believe so, too. A cold day, drafts, cold drinks or hot drinks of water set off tic. They might act similarly in setting off an attack in a tabetic.

DR. WEBSTER: After a few bad days, tabetics come flocking in for therapy of some kind.

DR. MCDERMOTT: Have you noticed any increase in the incidence of herpes zoster in tabetics who have been treated with penicillin?

DR. WEBSTER: I have not. Perhaps Dr. Peabody has.

DR. GEORGE E. PEABODY: I have seen only one case.

DR. MCDERMOTT: Every now and then a patient with tabes develops herpes zoster, particularly after a bad bout of pain. Early in the days of penicillin therapy the frequency of these cases seemed to have increased and the question arose whether it might be in some way related to an effect of the drug. I know of no evidence for it. I suppose there is nothing in it.

DR. WOLFF: After a severe bout of pain trigeminal tic is also sometimes followed by herpes.

DR. GEORGE READER: I wonder if Dr. Webster would comment on the danger of malarial therapy in tabes.

DR. WEBSTER: I believe all agree that there are no serious complications following penicillin therapy while, even under the best circumstances, there is a mortality of 3 to 5 per cent with malarial therapy for syphilis of the central nervous system. There is no doubt of the fact that penicillin therapy is easier to apply. Here we are inclined to

hospitalize the patients, but ambulatory treatment for syphilis of the central nervous system is feasible and is being carried out in other places, especially with procaine penicillin. Ambulatory treatment materially reduces the expense. When possible, or when there is reasonable possibility that one form of therapy is as effective as the other, penicillin is certainly preferable. Which of the two forms of treatment is the more effective is at present the basis of controversy. If penicillin does not turn out to be inferior, the preference for penicillin will be decisive.

DR. MCDERMOTT: How much does it cost for procaine penicillin in the treatment of a case of neurosyphilis?

DR. WEBSTER: It is about $70 for a course of therapy lasting fourteen days. In the ambulatory form of treatment the patient can go on working.

DR. MCDERMOTT: How long would the patient be out of work in treatment with malaria? What would be the cost there?

DR. WEBSTER: Each treatment with malaria represents an expenditure of several hundred dollars of someone's money. In this hospital I think our average period of hospitalization is at least three weeks in all cases of malaria; many stay longer. Then there is the period of convalescence. Federal and State Health authorities feel very strongly about the disadvantages of long hospitalization. This is why they are working so hard to develop methods of ambulatory treatment.

DR. WOLFF: Is there any danger of serious dysfunction in tabetics during treatment with malaria?

DR. MCDERMOTT: There certainly are. Manifestations of damage to proprioception are distinctly greater when the patient is put to bed. Even with the relatively benign therapeutic malaria which we use it is quite impossible to keep the patients up and about; they are too weak.

DR. WEBSTER: Gastric crises are precipitated by malaria. The lightning pains are very severe when precipitated either by artificial fever or malaria.

DR. WOLFF: How about the bladder?

DR. WEBSTER: In this hospital we have had several individuals in whom cord bladders developed during malarial therapy. The patient's vascular system is also an important consideration. Many of these patients are hypertensive. Hypertension is an added risk in malarial therapy.

DR. MCDERMOTT: Moreover, many of the neurosyphilitic patients have associated aortic syphilis. This is often asymptomatic and would permit the patient ten or fifteen years of active life. These patients might be thrown into cardiac failure by the severe shock of malaria. Would you agree with that?

DR. WEBSTER: Yes.

DR. WOLFF: Would the considerations of danger of malarial therapy be dismissed in the case of the paretic because of the seriousness of the disease?

DR. MCDERMOTT: My own belief, and I think Dr. Webster shares it, is that malarial therapy should be used in patients with primary optic atrophy, and in paresis, regardless of its hazards. In the other types it is my belief that malaria, being such a debilitating and potentially dangerous treatment, should not be used unless penicillin therapy has had a fair trial first.

SUMMARY

DR. HARRY GOLD: In volume 2 of the Cornell Conferences on Therapy, the treatment of neurosyphilis was included in a general discussion of the treatment of syphilis. That conference was held in 1946. There were indications of a break with traditional agents and plans of therapy. Arsphenamine, neoarsphenamine, mercury and tryparsamide were no longer in use in the New York Hospital. Although there was evidence of enthusiasm for the early experimental results with penicillin in neurosyphilis, a conservative line was advocated for routine treatment and this involved the use of arsenoxide for about a year with or without malarial fever. In the period of about four years which has elapsed, experience here has crystallized in

the form of a radical change in the treatment of syphilis of the nervous system. The metals have been abandoned. Penicillin has been adopted as the sole therapeutic agent. The duration of treatment has been reduced from one year to fourteen days during which the patient receives a single intramuscular dose of 300,000 units of procaine penicillin daily. This is applied in all forms of neurosyphilis, except in primary optic atrophy in which malarial fever therapy is used simultaneously. It is assumed from the current evidence that the 4.2 million units of penicillin given in this way is usually sufficient to destroy all the spirochetes, and what persists in the form of clinical symptoms or serologic abnormalities represents organic changes on which the antimicrobial agent can exert no influence. In view of the fact that uncertainties concerning this position still remain, the treatment is repeated if, after the first course, the clinical and serologic signs show unfavorable progression. In tabes or paresis, malarial fever therapy may be added.

The conference provided an opportunity to explore special problems which relate to the treatment of neurosyphilis: when the patient with syphilis is free of the danger of developing neurosyphilis; how long the patient with neurosyphilis needs to be under observation; the clinical and serologic criteria of cure; the behavior of syphilis acquired in the same exposure as gonorrhea; reasons for discordant results from different clinics; the special aspects of tabes, paresis and optic atrophy; the Herxheimer reaction; various forms of artificial fever and the dangers of malarial fever therapy.

Clinico-pathologic Conference

Hemochromatosis versus Addison's Disease*

STENOGRAPHIC reports, edited by Robert J. Glaser, M.D. and David E. Smith, Jr., M.D., of weekly clinico-pathologic conferences held in the Barnes Hospital, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, M. M. (No. 178445), was a white, married executive, fifty-four years of age, who entered the Barnes Hospital on November 22, 1949, because of diabetes, weight loss and swelling of the legs.

The family history was of interest only in that the patient's mother had died of "high blood pressure." When he was forty-one years of age the patient developed epigastric discomfort, and cholecystograms were said to have confirmed a diagnosis of "gallbladder disease." His symptoms gradually subsided. For many years he had taken iron periodically because of "anemia," and during this period he had been told by a physician that his blood pressure was low.

Seven years before entry the patient became impotent. Two years later he was found to have diabetes. About the same time he suffered severe sunburn; subsequently there had been a gradual increase in pigmentation of the skin. Four years prior to entry the patient was told that his basal metabolic rate was low.

His diabetes was controlled by diet and by 15 to 23 units of regular insulin daily during the five years which elapsed between the discovery of diabetes and his entry into the hospital. Several years before admission he began to have mild swelling of the ankles at night; during the six weeks immediately preceding admission the swelling had increased markedly and was associated with shortness of breath, cough and palpitation. He was given digitalis which he continued to take up to the time of admission. In addition, mercurial diuretics were given and resulted in a weight loss of 20 pounds. During the seven years from the onset of his first symptoms his weight decreased from 175 to 123 pounds.

At the time of entry physical examination revealed the patient's temperature to be 37.3°C., pulse 50, respirations 18 and blood pressure 90/70. He appeared much older than his stated age and was chronically ill. There was brownish pigmentation of the skin, particularly over the neck, forehead, hands, feet, and in the axillary and inguinal regions. There were prominent freckles over these areas. The skin was also dry and wrinkled and there was absence of axillary hair. No pigmentation of the mucous membranes was observed. Examination of the eyegrounds revealed slight sclerosis of the arterioles without exudate or hemorrhages. The upper respiratory tract appeared normal. There was no neck vein distention. Examination of the lungs revealed decreased breath sounds and decreased tactile fremitus at the right base. No rales were heard. The heart was slightly enlarged to the left. The rate varied between 36 and 50 with frequent coupling; the sounds were of good quality and there were no murmurs. The liver edge, which was felt 4 cm. below the right costal margin, was firm and thick but not tender. The prostate was normal. There was questionable pretibial edema. The remainder of the physical examination was within normal limits.

The laboratory findings were as follows: Blood count: red cells, 3,910,000; hemo-

* From the Departments of Internal Medicine and Pathology, Washington University School of Medicine and the Barnes Hospital, St. Louis, Mo.

globin, 13.4 gm.; hematocrit, 50 per cent; white cells, 4,600; differential count: basophiles 2 per cent; stab forms, 8 per cent; segmented forms, 39 per cent; lymphocytes, 47 per cent; monocytes, 4 per cent. Urinalysis: negative. Stool examination: negative. Blood Kahn test: negative. Blood chemistry: non-protein nitrogen, 28 mg. per cent; fasting blood sugar, 103 mg. per cent; total proteins, 7.6 gm. per cent; albumin, 5.0 gm. per cent; globulin, 2.6 gm. per cent; cephalin-cholesterol flocculation test, negative; thymol turbidity, 1.8 units; prothrombin time, 44 per cent of normal; bromsulfalein test, no retention in forty-five minutes; alkaline phosphatase, 4 Bodansky units; cholesterol, 131 mg. per cent; chlorides, 96 mEq./L.; CO_2 combining power, 35.8 mEq./L.; sodium, 143.4 mEq./L.; potassium, 5.1 mEq./L.; serum iron, 169 micrograms per cent; iron binding protein, 0. Basal metabolic rate: −17, −24 per cent. Venous pressure: 150 mm. NaCl. Circulation time (decholin): thirty-five seconds. Roentgenogram of the chest: The aortic and cardiac silhouettes were normal; a pleural effusion was present on the right. Flat film of the abdomen: negative. Skull films: normal. Electrocardiogram: Abnormal form of ventricular complex; low voltage and sinus bradycardia.

Shortly after the patient was admitted to the hospital intensive metabolic studies were begun. With a diet of 200 gm. of carbohydrate, 81 gm. of protein and 100 gm. of fat, his diabetes was well regulated on a mixture of 10 units of protamine and 20 units of regular insulin. A Robinson-Power-Kepler water test was positive. The patient was then subjected to a Cutler-Wilder test during which he grew progressively weaker but no change in blood sodium, potassium, chloride or CO_2 combining power occurred, and the test was considered negative. During this period of the test, however, he had several severe insulin reactions necessitating reduction in the total amount of insulin administered. A Thorn test with epinephrine revealed no fall in eosinophils but there was a 54 per cent reduction in eosinophils after ACTH. Twenty-four hour excretion studies of various steroid hormones revealed low to low normal values for cortin, 17-ketosteroids, follicular stimulating hormone, estrogens and androgens.

Because of the presence of diabetes in a patient with increased skin pigmentation the diagnosis of hemochromatosis was considered and a skin biopsy was performed. Histologic section revealed iron pigment identified as hemosiderin. Hemosiderin granules were also found in cells and in casts in the urinary sediment. A needle biopsy section of the liver showed fibrous tissue, an increase in the number of bile ducts and large amounts of hemosiderin.

Beginning at the end of the second hospital week the patient was given a regimen which included 5 mg. of DOCA and 10 gm. of extra dietary salt daily. During the nine days he received this therapy he gained 10 pounds in weight; his blood pressure, however, did not rise significantly and he developed no obvious edema. DOCA was then omitted, the salt intake was reduced and the patient was given testosterone and a dietary regimen aimed at treatment of his liver disease. He continued to gain weight; in addition, dyspnea, neck vein distention, basal rales, increase in the right pleural effusion, marked hepatomegaly and increasing ankle edema were noted. Mercurial diuretics and larger amounts of digitalis were given, but the patient's response was poor and he became nauseated.

At the end of the third hospital week he developed Cheyne-Stokes respirations and his urinary output became markedly reduced. The signs of heart failure were not improved by further use of mercurial diuretics. Periods of coupling were noted during which the apical rate was 88 and the radial pulse rate 44. Digitalis was temporarily discontinued but two days later the patient developed rapid auricular fibrillation and was given frequent small doses of lanatoside C intravenously. The cardiac rhythm reverted to a sinus mechanism but soon after coupling reappeared along with multifocal ventricular premature contrac-

tions. The latter were controlled with small doses of quinidine.

A thoracentesis was performed and 1,100 cc. of orange-brown fluid were removed from the right pleural cavity; the fluid had the characteristics of a transudate. Repeat blood chemical studies revealed the following data: Non-protein nitrogen, 21 mg. per cent; sodium, 136.6 mEq./L.; potassium, 4.6 mEq./L.; chloride, 89 mEq./L.; CO_2 combining power, 33 mEq./L.; total proteins, 6.5 gm. per cent; albumin, 4.4 gm. per cent; globulin, 2.1 gm. per cent; hematocrit, 36 per cent.

Testosterone therapy was stopped and eventually the patient exhibited a moderately satisfactory diuresis; concomitantly his appetite increased. Five days before death, however, nausea, which had been intermittent, increased, as did edema; the signs of marked ascites were noted and the patient's urinary output again fell. Eventually he became anuric and irrational. The following laboratory data were then recorded: non-protein nitrogen, 100 mg. per cent; sugar, 611 mg. per cent; chloride, 83 mEq./L.; CO_2 combining power, 25.6 mEq./L.; cephalin-cholesterol flocculation test, 3+; thymol turbidity test, 3.5 units; icterus index, 21 units; alkaline phosphatase, 8 Bodansky units; total proteins, 6.1 gm. per cent; total bilirubin, 1.32 mg. per cent; prothrombin time, 18 per cent of normal.

Intractable cardiac failure persisted and the patient died quietly on December 25, 1949.

CLINICAL DISCUSSION

DR. W. BARRY WOOD, JR.: This case is a most complicated one. In our discussion I believe we may best approach the problems presented by first considering the two diagnostic possibilities considered most seriously in the hospital—primary adrenal insufficiency and hemochromatosis. We shall attempt to explain as many of the symptoms, signs and laboratory findings as possible on one or the other of these two possibilities, and then see which seems the more likely of the two.

This patient was carefully studied under Dr. Olmsted's direction and we, therefore, have much valuable material on which to base our conclusions. It should be possible for us to reach a correct diagnosis, localize the lesions and predict what the pathologist will find. Let us begin by considering first the possibility which I believe was entertained when the patient was admitted to the hospital, namely, that he had some form of Addison's disease. Dr. MacBryde, how many of the various findings in regard to the history (Table I) are compatible with the diagnosis of Addison's disease?

TABLE I
HISTORY

Symptom	Addison's	Hemochromatosis
Hypotension	+	+
Hypometabolism	+	+
Diabetes	coincidental	+
Impotence	+	+
Hyperpigmentation	+	+
Dyspnea and edema	0	+
Weight loss	+	+

+ = compatible 0 = incompatible

DR. CYRIL M. MACBRYDE: Certainly hypotension, hypometabolism, impotence, pigmentation and weight loss are consistent with the diagnosis of Addison's disease. Diabetes may occur coincidentally in a patient with Addison's disease; when it does develop certain changes in the basic clinical picture of Addison's disease often occur. Dyspnea and edema cannot be explained on the basis of untreated Addison's disease.

DR. WOOD: In regard to the physical examination (Table II) how many of the findings are compatible with Addison's disease?

DR. MACBRYDE: Again hypotension, hyperpigmentation and sparsity of hair are common in Addison's disease. Cardiac enlargement, bradycardia, pleural effusion and hepatomegaly cannot be considered typical of untreated Addison's disease.

DR. WOOD: Can the pigmentation of hemochromatosis be differentiated from that of Addison's disease on inspection?

DR. MACBRYDE: Differentiation is often very difficult although there are certain differences which may be helpful. In hemochromatosis the pigmentation is frequently diffuse and may have a bluish tinge whereas in Addison's disease the pigmentation is beige or brown. Mucous membrane pigmentation suggests adrenal cortical insufficiency.

TABLE II
PHYSICAL FINDINGS

Sign	Addison's	Hemochromatosis
Hypotension	+	+
Hyperpigmentation	+	+
Sparsity of hair	+	+
Cardiac enlargement	0	+
Bradycardia	0	0
Pleural effusion	0	+
Hepatomegaly	0	+

+ = compatible 0 = incompatible

DR. WOOD: You have indicated that cardiac enlargement is not consistent with Addison's disease.

DR. MACBRYDE: Characteristically, the heart is small in adrenal insufficiency unless the patient is overtreated with DOCA.

DR. WOOD: I believe that Dr. Olmsted pointed out, shortly after this patient was admitted to the hospital, that hepatomegaly was not consistent with the diagnosis of adrenal insufficiency, and I take it, Dr. MacBryde, from what you have said that you agree.

DR. MACBRYDE: Yes.

DR. WOOD: The laboratory studies (Table III) were rather extensive, and should be quite helpful. Dr. Hunter, would you comment on the results. Is anemia common in adrenal insufficiency?

DR. THOMAS H. HUNTER: Mild anemia is compatible with that diagnosis.

DR. WOOD: What about lymphocytosis?

DR. HUNTER: Increase in lymphocytes is rather typical.

DR. WOOD: The blood sugar was 103 mg. per cent fasting, when the patient entered the hospital, and later was recorded as high as 630 mg. per cent.

DR. HUNTER: In Addison's disease, *per se* a low normal blood sugar or hypoglycemia is the rule. The elevated blood sugar certainly is due here to the concomitant occurrence of diabetes.

TABLE III
GENERAL LABORATORY DATA

Test	Addison's	Hemochromatosis
Anemia	+	+
Lymphocytosis	+	+
Elevated blood sugar	0	+
Rising NPN	+	+
CO_2 combining power 35.8–25.6 mEq./L	?	?
Chloride 96–83 mEq./L	+	?
Sodium 143–136 mEq./L	+	?
Cholesterol 135 mg. per cent	?	+
Serum bilirubin 1.3 mg. per cent	0	+
Prothrombin time low	0	+
Cephalin flocculation test 0–3+	0	+
BMR −17, −24 per cent	+	+

+ = compatible; 0 = incompatible; ? = not known.

DR. WOOD: What about an elevated nonprotein nitrogen along with an increase in carbon dioxide combining power and a normal serum chloride.

DR. HUNTER: Slight elevation in the nonprotein nitrogen may be seen in patients with adrenal insufficiency, particularly those in crisis. The elevated carbon dioxide combining power in the presence of a normal chloride seems unusual to me and I cannot explain it. The subsequent fall in chloride would be compatible with adrenal insufficiency. When the chlorides are low the carbon dioxide combining power may rise as a compensatory measure, but it was highest here when the chlorides were nearest normal.

DR. WOOD: I agree that the finding of a high CO_2 combining power in this man at the time that his serum chloride was normal is a very puzzling one. Dr. MacBryde, do you have an explanation for it?

DR. MACBRYDE: No. I do not.

DR. HUNTER: To go on, a decrease in sodium is characteristic of Addison's disease. I am not aware that a low cholesterol has any significance.

DR. WOOD: Dr. MacBryde, is there any information in the literature on that point?

TABLE IV
SPECIAL LABORATORY DATA

	Addison's	Hemochromatosis
Thorn test		
Epinephrine = 18%	+	+
ACTH = 54%	±	±
Positive Kepler-Power test	+	+
Negative Cutler-Wilder test	0	±
Urinary steroids		
Cortin normal	0	?
17-Ks low	+	+
Androgens low	+	+
Estrogens low	+	+
FSH low	+	+
No iron-binding protein	0	+
Elevated serum iron	0	+
Hemosiderin in skin	0	+
Hemosiderin in liver	0	+
Hemosiderin in casts	0	+

+ = compatible ± = equivocal
0 = incompatible ? = not known

DR. MACBRYDE: I am not aware of any, but when the cholesterol is extremely low in Addison's disease one should suspect some other factor.

DR. HUNTER: The low BMR is certainly consistent with the diagnosis of Addison's disease, but I do not believe that elevation of the serum bilirubin, decrease in the prothrombin time, and the abnormal cephalin-cholesterol flocculation test are helpful.

DR. WOOD: Certain special tests were undertaken in this patient (Table IV). Dr. Glaser, would you comment on the Thorn test? It was done with both epinephrine and ACTH.

DR. ROBERT J. GLASER: If one gives a normal patient epinephrine, the pituitary is stimulated and in turn its secretion stimulates the adrenal cortex, activity of which is manifested by a fall in circulating eosinophiles. If, on the other hand, ACTH is given the adrenals are stimulated directly. In Addison's disease the test should be negative both with epinephrine and ACTH. In this patient there was no effect from epinephrine, suggesting that the pituitary did not respond to the stimulation of epinephrine. The 54 per cent fall with ACTH represents a minimal response, I believe.

DR. MACBRYDE: It is well to point out that this patient received a rather large amount of ACTH. The response of the adrenal cortex probably varies with the degree of stimulation. Thus, enough ACTH may be given to certain patients with proven adrenocortical insufficiency to lead to some response.

DR. WOOD: How do you interpret the results of these procedures, Dr. MacBryde?

DR. MACBRYDE: I agree with Dr. Glaser that the pituitary exhibited no response to epinephrine. The response to ACTH does not rule out adrenocortical insufficiency.

DR. WOOD: The tests, however, suggest that the pituitary has relatively poorer function than the adrenal. Do you agree?

DR. MACBRYDE: I believe so.

DR. WOOD: In that case one might make a diagnosis of adrenal insufficiency secondary to hypopituitarism. I believe it is said that patients whose adrenal insufficiency is secondary to pituitary disease do not exhibit abnormal pigmentation. Do you subscribe to that concept, Dr. MacBryde?

DR. MACBRYDE: No. I do not. I believe that they may or may not have abnormal pigmentation. I have seen at least two patients with Simmonds' disease who also exhibited the typical pigmentation of Addison's disease.

DR. WOOD: It is interesting that Dr. Thorn states that hyperpigmentation rarely results from involvement of the pituitary alone and that its presence in a patient with signs of hypopituitarism suggests involvement of the adrenal as well as the hypophysis.* It is probably fair to say that pigmentation is more common if the disturbance is primarily in the adrenal rather than in the pituitary.

* THORN, G. W. The Diagnosis and Treatment of Adrenal Insufficiency, p. 20. Springfield, 1949. Charles C Thomas.

In regard to the diagnosis of adrenal insufficiency it is of interest to note that the Kepler-Power water test was positive but that the Cutler-Wilder test was negative. Would you comment on those findings, Dr. MacBryde.

DR. MACBRYDE: I would have predicted *a priori* that the patient would have had a positive Cutler-Wilder test. In this patient there were many complicating factors and the test had to be interpreted as being negative.

DR. CARL L. COOK: Other than the episode of hypoglycemia the patient got along very well during the performance of the Cutler-Wilder test.

DR. WOOD: Certainly a negative Cutler-Wilder test is strongly against the diagnosis of Addison's disease. The various urinary steroid determinations should be helpful in arriving at the correct diagnosis. Dr. MacBryde, would you comment on those results.

DR. MACBRYDE: The normal cortin excretion is against the diagnosis of Addison's disease, but the results of the other four determinations are compatible with it.

DR. WOOD: In passing it should be noted that the patient's apparent sensitivity to insulin would be in keeping either with the diagnosis of adrenal insufficiency or of pituitary insufficiency. On the basis of the various data available to us there is already much to suggest that the patient's adrenal insufficiency was secondary to a disturbance of the pituitary; it is, of course, possible that both the pituitary and adrenal will show signs of involvement. Let us now consider hemochromatosis, the diagnosis in the second column in our four tables. I should like to ask Dr. Carl Moore if he will begin this aspect of the discussion.

DR. CARL V. MOORE: In regard to the history all the items listed in Table 1 are consistent with the diagnosis of hemochromatosis, although all of them are not especially characteristic. Certainly diabetes, impotence and pigmentation are typical and dyspnea and edema may be related to the cardiac failure which often occurs in hemochromatosis. As for the physical findings, all of those listed with the exception of bradycardia are consistent with hemochromatosis. Hepatic enlargement is particularly common.

Of the various general laboratory data some of the results are compatible with hemochromatosis, while others are explicable on the basis of the complicating cardiac failure. Slight anemia may be seen. An elevated blood sugar is, of course, common and the non-protein nitrogen may be elevated. I am unaware that the values for CO_2 combining power, chloride and sodium suggest hemochromatosis; on the other hand, I do not believe the results are against the diagnosis. A low basal metabolic rate is not characteristic of hemochromatosis, but it is not incompatible with it. As far as the special procedures are concerned, the result of the Thorn test is quite in keeping with hemochromatosis which has involved the pituitary. Perhaps Dr. MacBryde will comment on the results of the metabolic studies.

DR. MACBRYDE: As I indicated when we were considering adrenal insufficiency, it is difficult to interpret the positive Kepler-Power test and the negative Cutler-Wilder test. As far as I know cortin values have not been determined previously in patients with hemochromatosis. The decreased excretion of the other four types of steroids is entirely compatible.

DR. MOORE: The absence of iron-binding protein and the high serum iron value are typical of hemochromatosis. Likewise the demonstration of hemosiderin in the skin and in the liver, and the presence of hemosiderin casts in the urine, are all strongly in favor of the diagnosis.

DR. WOOD: Would you comment further on the iron-binding protein and the serum iron levels?

DR. MOORE: Apparently iron is carried in the plasma in combination with a beta-1 globulin which has been obtained in crystalline form in Dr. Cohn's laboratory at Harvard. Normally the serum iron varies from about 60 to 180 micrograms per 100 cc. In addition there is enough iron-binding

protein available so that it can combine with another 100 or 150 micrograms of iron if that much is present. It is quite characteristic in hemochromatosis and in hemosiderosis that all of the iron-binding protein is saturated with iron. Thus although in this patient the serum iron value was not as high as some which have been recorded in hemochromatosis, there was no capacity for further binding of iron. It is of great interest that this observation is typical not only of patients with hemochromatosis but also may be demonstrated in relatives of such patients even though such relatives do not have clinical hemochromatosis.

DR. WOOD: It seems clear then that this patient probably had secondary adrenal insufficiency due to hemochromatosis which involved the pituitary gland. Dr. Olmsted, would you predict what findings the pathologist will report?

DR. WILLIAM OLMSTED: Certainly this man will have large amounts of iron in his liver and in other organs of his body. There are cases recorded in the literature in which the pituitary has been completely destroyed in hemochromatosis. In view of his impotence it would not be surprising if the testes were also involved by the process. The patient died of heart failure and it may well be that the myocardium will show large amounts of iron. In that respect the use of DOCA was contraindicated in this patient.

DR. WOOD: On the other hand, you were in a very difficult position because of the signs of adrenal insufficiency which this patient exhibited.

DR. OLMSTED: I am sure large amounts of iron will be found in the pancreas; and in the liver, in addition to the presence of iron, cirrhosis may also be found.

DR. WOOD: Mr. Elmer Brown of the senior class has been working on the problem of iron metabolism in Dr. Carl Moore's laboratory, and I am going to ask him to comment on his concept of this disease.

MR. ELMER B. BROWN, JR.: It seems likely that the basic defect in hemochromatosis is the increased absorption of iron from the gastrointestinal tract, although increased absorption alone could probably not account for all of the manifestations of the disease. After reviewing many theories as to the pathogenesis of hemochromatosis, Sheldon came to the conclusion that the disease is an inborn error of metabolism; that explanation seems most tenable to me for it best explains the predominance of the disease in males, the fairly well documented familial cases, the greatly increased iron stores with diabetes, cirrhosis and skin pigmentation, the other pigment abnormalities and the metabolic changes which have been reported.

Sheldon postulated that the excess iron accumulates because of increased iron absorption from the intestine over a long period of time; he calculated that the time necessary for this to occur is approximately twenty-eight years in the average case of hemochromatosis. It seems obvious that the ultimate source for this iron could only be from increased absorption though many early investigators postulated that the iron came from a breakdown of the red blood cells or a redistribution or lack of neutralization of body iron. This theory could not account for the massive increase in body iron stores. Many balance studies have been performed to see whether patients with hemochromatosis absorbed more iron than normal people; the results of these studies are equivocal, probably because of the difficulties inherent in the measurement of iron intake and excretion. However, Dr. Moore and his associates used radioactive iron to measure the absorption of iron in one patient with hemochromatosis and they found greater than normal iron absorption with most of the iron going to the tissue stores. This technic has not yet been used extensively, hence, this evidence, though suggestive, is not conclusive.

It is a widely held clinical impression that the excessive iron storage in the liver, pancreas and other organs in hemochromatosis leads to the development of fibrosis and cirrhosis of these organs. There is abundant experimental work, however, which fails to demonstrate any fibrosis as the result of pro-

longed iron deposition in animals. Further, the fact that patients receiving large amounts of iron over long periods by transfusions frequently develop fibrosis and cirrhosis does not mean that the iron in the tissues is responsible for the pathologic changes. In such cases one has the added variables of the anemia which necessitated the transfusions, frequent febrile transfusion reactions with damage especially to the liver, and the ever present danger of homologous serum hepatitis from the multiple transfusions. I do not know what the cause for the cirrhosis and diabetes is in hemochromatosis, but I am inclined to believe that it is just another aspect of the inborn error of metabolism—a tendency to increased fibrous tissue proliferation, possibly accentuated by the increased iron deposits. There is, however, no evidence for such a postulation.

DR. WOOD: There is involvement of several other pigments in hemochromatosis, is there not?

MR. BROWN: Yes. While the most common pigment seen in the tissues, especially in the liver, is hemosiderin, the iron-containing pigment staining blue with the ferrocyanide reaction, there is also a non-iron-containing pigment, hemofuscin, which stains red with basic fuchsin. As far as I know, there is not much known about this pigment except that it is a lipoid substance which is found quite constantly in hemochromatosis, chiefly in the liver, spleen and pancreas and especially in the walls of the small blood vessels. Many investigators believe hemofuscin is the same as or similar to the brown pigment of atrophy found in normal old people. There has also been shown to be an increased amount of copper, sulfur and calcium in the tissues of patients with hemochromatosis.

DR. WOOD: Melanin is increased in the skin, is it not?

MR. BROWN: This finding is apparently variable; often there is a considerable increase, enough to obscure the blue color produced by injected potassium ferrocyanide in the deeper dermal layers—one of the skin tests for hemochromatosis. In other cases there may be no more than normal amounts of melanin in the skin. The variation has been attributed to the variable involvement of the adrenals by iron deposit there.

DR. WOOD: Then this disease can hardly be explained simply by increased iron absorption?

MR. BROWN: I believe not.

DR. WOOD: Is there any part of the world in which this disease is common?

MR. BROWN: I am not sure that the disease described by the Gilmans in South African natives with chronic pellagra is true hemochromatosis; in fact, I do not believe it is for it differs from typical hemochromatosis in a number of ways: the disease comes on at a much earlier age (less than forty years usually); it has an equal sex distribution; it is definitely related to diet; there is no reported associated diabetes and it is extremely frequent. But the Gilmans have reported a very widespread incidence of iron pigmentation and cirrhosis in these natives who eat a corn grit diet.

DR. WOOD: What is thought to be the explanation for the iron deposition in these people?

MR. BROWN: Stimulated by the reports of these South African investigators, Rath and Finch have fed rats a corn grit diet similar to that of the pellagnous natives and have observed a moderate increase in iron deposition in the liver and other organs of these animals. Further experiments have shown that when iron is added to the corn grit diet there is greatly increased absorption of iron, and large amounts are deposited in the liver. It seems that the low phosphorus content of this corn grit diet is the factor which allows increased iron absorption since one can prevent the increased iron absorption by adding phosphates to this diet. Apparently then, in the natives eating a corn grit diet with a low phosphorus content, increased amounts of iron are absorbed, the source of which has not been found yet though it may be in the food, drinking water or from iron utensils used in cooking. There is no suggestion, however, that such a mechanism can explain the

hemochromatosis seen in today's patient and we must fall back on the rather vague postulate of an inborn error of metabolism.

DR. WOOD: Thank you, Mr. Brown. I think there is good agreement with Dr. Olmsted's original clinical diagnosis, namely, that this patient had hemochromatosis with cardiac involvement and with polyglandular deficiency due primarily to destruction of the hypophysis.

Clinical Diagnoses: Hemochromatosis with pituitary involvement and cardiac insufficiency.

PATHOLOGIC DISCUSSION

DR. WILLIAM T. SNODDY: The skin was light brown over most of the body surface, and brown in the inguinal and axillary regions. No axillary hair was present. All the serous cavities contained cloudy yellow fluid—the peritoneal cavity 1,850 ml., the right pleural cavity 1,350 ml., and the left pleural cavity 1,000 ml. The heart was slightly increased in size. Its chambers were dilated, and the myocardium was flabby and distinctly browner than usual. The lungs were remarkable only for partial atelectasis, particularly of the lower lobes, and slight evidence of congestion and edema. The liver was enlarged to 2,430 gm. and was very firm, rusty red and slightly nodular. On section the lobular architecture was preserved and accentuated by connective tissue. The pancreas was firm and dark rusty red but its configuration and architecture were not abnormal. The spleen was slightly enlarged, dark red and firm; its cut surfaces did not bulge above the capsule. The testicular stroma was firm and decreased in amount, and the seminiferous tubules did not string from the cut surfaces. All other organs were considered to be of essentially normal gross appearance.

DR. GUSTAVE J. DAMMIN: Without reproduction in color to show the intense blue of the sections stained for iron, the illustrations do not adequately convey the striking extent of deposition of iron pigment in the various tissues. Figure 1 is of a section of the liver. The coarse granularity of the hepatic cells is due to the presence of large amounts of hemosiderin in their cytoplasm. There is also an increase of fibrous tissue in the portal triads and atrophy of the hepatic cells in the central portions of the lobules, but the outlines of the lobular architecture are preserved. Central veins are present in their usual sites, and none of the large nodules of regenerated hepatic parenchyma typical of the usual portal cirrhosis is seen. In other fields a slightly increased number of bile ducts, granules of iron in the epithelium of the bile ducts and deposits of hemofuscin about blood vessels were demonstrable. A similar process involves the pancreas (Fig. 2) in which there is a diffuse increase of interstitial fibrous tissue and an absolute reduction in the amount of glandular epithelium despite the apparent normal size of the organ grossly. Large amounts of iron are present in the epithelium of the acini and lesser amounts in that of the pancreatic ducts and islets and in the interstitial connective tissue. Islets of Langerhans are easily identified and their architecture is changed only by the presence of columnar metaplasia of the cells and hyperplasia in some islets.

The myocardium manifested several changes. There was a diffuse increase of interstitial connective tissue throughout the sections with large amounts of fat present in the interstitial tissue and within vacuoles in the myocardial fibers. The latter change and the presence of paranuclear granules of hemosiderin and hemofuscin are illustrated in the highly magnified section in Figure 3. Where the granules of iron are particularly numerous, the intracellular fibrils are displaced or destroyed by their presence. It is not generally agreed whether or not the iron itself is responsible for the changes in the myocardium in hemochromatosis, but regardless of the mechanism of injury, the cardiac musculature in this case showed signs of diffuse and severe damage.

We were particularly interested in evaluating the degree of involvement of the adrenal glands. Grossly, they were of normal appearance but without special procedures

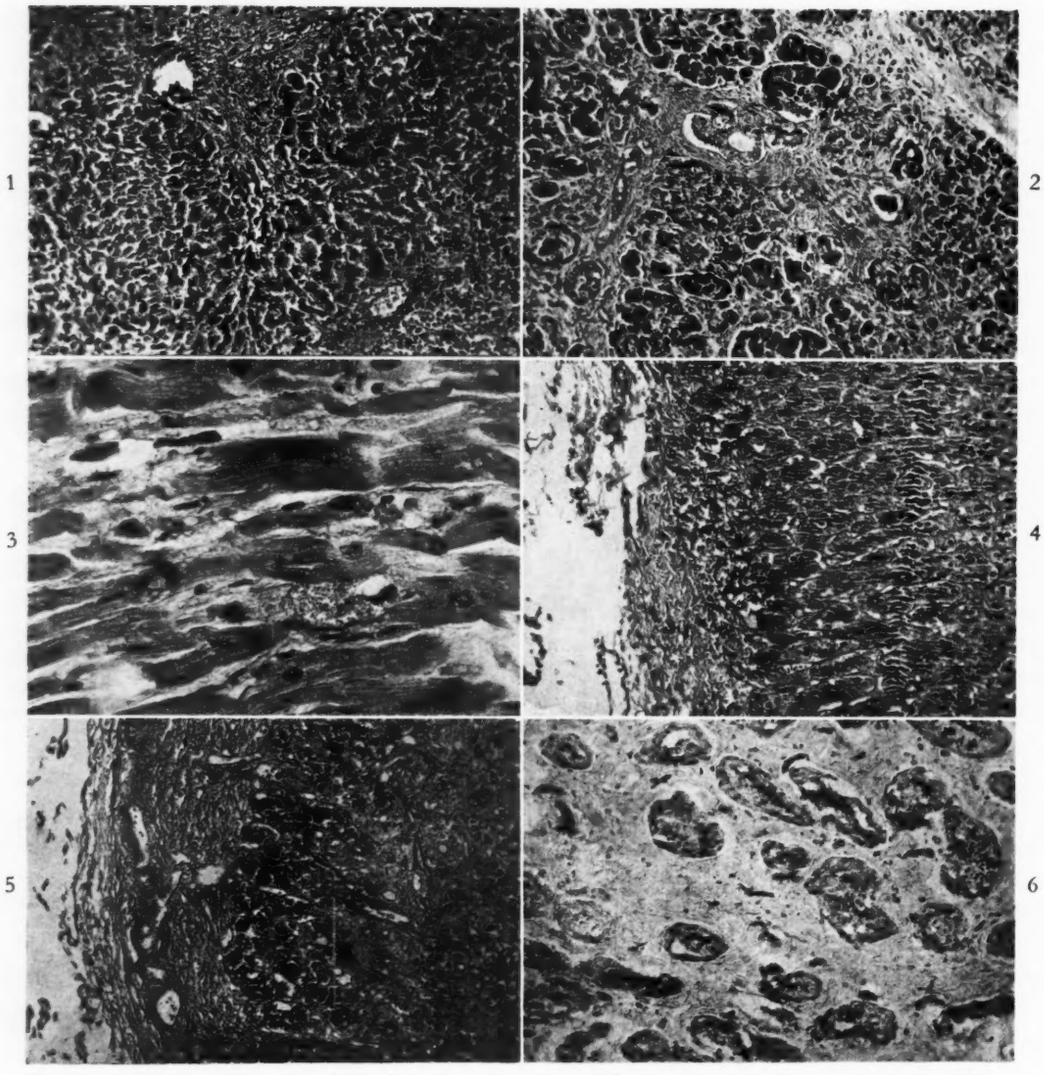

FIG. 1. A section of liver in which there is an increase of fibrous tissue in the portal triads without destruction or regeneration of the lobules. The granularity of the hepatic cells is due to the presence of hemosiderin, and the central atrophy in the lower right corner is secondary to congestive failure.
FIG. 2. Interstitial fibrosis and atrophy in the pancreas. The large amount of hemosiderin in the epithelium is not adequately represented without color.
FIG. 3. Paranuclear hemosiderin and hemofuscin in the myocardium. There is also a fine vacuolation of portions of some fibers due to the presence of fat.
FIG. 4. The adrenal with a small amount of hemosiderin in the outermost cells of the cortex.
FIG. 5. Edge of the pituitary with a narrow zone of preserved epithelial cells and extensive fibrosis beginning at the right of the section. The fibrous tissue replaced more than two-thirds of the gland.
FIG. 6. Interstitial fibrosis and atrophy of the seminiferous tubules in the testis. Practically no iron is present in sections of this organ.

it would be impossible to make a very significant estimate of the functional activity of that organ. In a general manner, however, the content of fat is a fairly reliable index of the functional integrity of the adrenal; and stains for fat on sections of these organs demonstrated large amounts of sudanophilic material. The only difference from normal is a decrease of fat in the outer layer of the cortex where there are also the only deposits of hemosiderin in this gland. (Fig. 4.) Similarly, the other major viscera were but little altered histologically. The spleen had a slightly thickened capsule, and trabeculae and sinusoidal walls that suggested a very slight degree of portal hypertension, proba-

bly secondary to cirrhosis and cardiac failure. Hemosiderin was present in that organ in practically insignificant amounts. In the kidney there were a few granules of hemosiderin in some distal convoluted tubules and congestion commensurate with the other evidences of terminal cardiac decompensation. Sections of the bone marrow were interesting for they contained large amounts of iron without an increase in the amount of fibrous tissue or disturbance in the cellularity. This is a particularly significant feature of hemochromatosis which, with additional evidence derived from the experimental administration of iron to animals, suggests that the presence alone of excess iron in the tissues is not responsible for the interstitial fibrosis characteristically present in the liver, pancreas and heart.

Portions of many organs were analyzed in Dr. Carl Moore's laboratory for their content of iron. The calculated total content of the liver was 23.6 gm., of the spleen 1.2 gm., the kidneys 0.2 gm., the pancreas 1.36 gm and the heart 2.0 gm. In these five organs alone there were 28.36 gm. of iron. The normal amount for the whole body is between 1 and 3 gm.; thus at least nine or ten times the usual amount of iron was demonstrated without consideration of the iron in the skin, lymph nodes, muscle or other tissues, all of which probably contained significant amounts.

The pituitary exhibited changes which were probably responsible for many of the clinical evidences of disturbed endocrine functions. More than two-thirds of all sections of this gland were composed of fibrous tissue and the epithelial cells of the anterior lobe were reduced to small groups. Figure 5 represents a typical area near the capsule. Large amounts of iron are present in the fibrous tissue as well as in the epithelial cells. Among the latter the eosinophilic and basophilic cells were present in about their normal numerical ratios, but there appeared to be a reduction in the number of chromophobic cells. As reported by some, the iron appeared to be concentrated in the basophilic cells.

A section of the testis (Fig. 6) shows what is probably an effect secondary to the changes in the pituitary. There is profound atrophy of the seminiferous tubules and increased interstitial fibrous tissue without significant deposition of iron at any site in the organ.

In summary, the gross and microscopic changes were typical of hemochromatosis with interstitial fibrosis in the liver, pancreas, heart and pituitary gland. Involvement of the latter was probably responsible for the long-standing evidences of pituitary deficiency described clinically. The involvement of the myocardium, regardless of the exact mechanism by which it occurred, led to cardiac decompensation and the changes of congestion and accumulation of fluid indicative of the myocardial failure which were directly responsible for the patient's death.

Final Anatomic Diagnoses: Hemochromatosis of the liver, pancreas, pituitary, skin, myocardium bone marrow, adrenals, intestinal mucosa, kidneys and spleen; cirrhosis of the liver, moderate; interstitial fibrosis of the pancreas; atrophy and fibrosis of the pituitary; interstitial fibrosis, fatty degeneration and focal atrophy of the myocardium; congestion of the lungs, liver, spleen and kidneys.

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Special Feature

Southern Society for Clinical Research

ABSTRACTS OF PAPERS PRESENTED AT THE FOURTH ANNUAL MEETING, NEW ORLEANS, LOUISIANA, MARCH 18, 1950

QUANTITATIVE DETERMINATION OF GLOBIN, HEMOGLOBIN AND SERUM PROTEINS IN ANEMIC AND NON-ANEMIC PATIENTS. *Walter Lyon Bloom, M.D., J. G. Palmer, M.D. and George T. Lewis, M.D. (introduced by Arthur J. Merrill, M.D.). Atlanta, Ga.* (From the Departments of Medicine and Biochemistry, Emory University and Medical Division, Lawson Veterans Administration Hospital.)

Methods for study of erythrocyte pigment and iron are well established. Previously no simple quantitative method for the determination of erythrocyte proteins has been available even though globin constitutes 96 per cent of hemoglobin. A quantitative method for the determination of erythrocyte proteins is presented in this paper. Micro-Kjeldahl analysis was used for all nitrogen determinations. The total erythrocyte nitrogen, globin nitrogen and nonprotein nitrogen were studies on centrifuged washed cells from 4 cc. of blood. Hemoglobin was estimated by manometric and spectrophotometric methods on nineteen patients.

The globin concentration per 100 cc. of blood is found to agree with the amount of hemoglobin determined by the manometric and spectrophotometric methods. Nine of the nineteen patients had hemoglobin concentrations below 14 gm. per cent. Only one of these nine patients was found to have a diminished erythrocyte protein. This change was found to agree with hypochromia in this patient. Simultaneous serum protein determinations on all patients emphasized that in the absence of anemia there is approximately five times more cell protein than serum protein in 100 cc. of whole blood. This method provides a means of determining hemoglobin as well as studying certain aspects of cell protein metabolism and the relation of erythrocyte protein to anemia.

BROMSULPHTHALEIN AS A TOOL FOR THE STUDY OF LIVER PHYSIOLOGY. *R. W. Brauer, M.D., J. S. Krebs, M.D. (by invitation) and R. L. Pessotti, M.D. (by invitation). New Orleans, La.* (From the Departments of Pharmacology and Radiobiology, Louisiana State University School of Medicine.)

Bromsulphthalein (BSP) uptake from the blood stream in dogs was studied by means of the continuous infusion technic. Steady levels of blood and bile BSP can be attained by this means. Dye extraction at infusion rates below 0.2 mg./kg./min. occurs at rates near 12 per cent of circulating dye per minute. With further increase of infusion rate a rapid decrease of extraction efficiency is observed. Bile collection during such infusion has shown: (1) Colorimetrically determined BSP concentrations of 1,000 mg. per cent represent the limit attainable in the dog. (2) Colorimetric BSP excretion occurs at rates from 30 to 70 per cent of infusion rates. Analysis of livers in such experiments shows that a considerable amount of BSP remains unaccounted for while some BSP accumulation occurs in the liver. Using S^{35} tagged BSP under similar conditions, nearly quantitative recovery of S^{35} infused can be made from liver, blood and bile. Chromatographic analysis of bile BSP shows that S^{35} can be recovered in four fractions, different from BSP, none of them colorless. The significance of these findings in connection with the basic mechanisms of BSP excretion and the question of extrahepatic BSP uptake will be considered.

ADRENOCORTICAL FUNCTION IN ESSENTIAL HYPERTENSION: A STUDY OF SWEAT SODIUM CONCENTRATION. *Rod M. Buie, Jr., M.D., Seymour Eisenberg, M.D. and Louis Tobian, Jr., M.D. (introduced by Tinsley R. Harrison, M.D.). Dallas, Tex.* (From the Department of Medicine, Southwestern Medical College.)

Arterial hypertension often results from prolonged adrenocortical hypersecretion, a possible factor in patients with "essential" hypertension. Since hypersecretion of "electrolyte influencing"

adrenocortical steroids causes a prolonged decrease in sweat sodium concentration, this determination was performed in essential hypertensives with the following results:

Group	Average Blood Pressure mm. Hg	Mean Sweat Sodium Concentration (mEq./L.) and Standard Deviation
33 Normotensives	124/74	38.2 (S.D. ± 18.2)
35 Hypertensives	203/122	44.0 (S.D. ± 25.6)
11 Hypertensives with diastolic blood pressure between 100 and 124 mm. Hg	228/139	33.7 (S.D. ± 26.1)
24 Hypertensives with diastolic blood pressure between 100 and 124 mm. Hg	194/114	49.5 (S.D. ± 25.3)

There is no significant difference between any of these groups. This makes it very unlikely that ordinary essential hypertensives have any considerable increase in the level of "electrolyte influencing" adrenocortical steroids.

DESOXYCORTICOSTERONE AND THE URINARY EXCRETION OF SODIUM CHLORIDE. *Walter H. Cargill, M.D. and Herbert A. St. John, M.D. (introduced by James V. Warren, M.D.). Atlanta, Ga.* (From the Department of Physiology, Emory University, and The Medical Service, Lawson Veterans Administration Hospital.)

The effects of desoxycorticosterone acetate (DCA) and desoxycorticosterone glucoside (DCG) on the urinary elimination of a salt load imposed by the intravenous infusion of hypertonic (4 per cent) saline have been studied. Soffer's observation of an increased rate of salt excretion following DCA administration in Cushing's syndrome has been confirmed in five patients. A similar reversal of the usual action of DCA has been demonstrated in two patients with rheumatoid arthritis receiving pituitary adrenocorticotrophic hormone. Measurement of glomerular filtration rate and renal plasma flow by inulin and sodium para-aminohippurate clearances revealed that the observed changes in salt excretion resulted entirely from alterations in the tubular reabsorption of sodium and chloride. Two patients with diabetes insipidus showed increased sensitivity to the salt-retaining action of DCA. The intravenous injection of DCG had no demonstrable effect on salt excretion or renal function in any of these subjects. These experiments indicate that under certain conditions of adrenocortical hyperactivity a paradoxic action of DCA on salt excretion may be observed. Advantage has been taken of this fact to study the role of the adrenal cortex in the renal regulation of salt balance when salt intake is reduced. Preliminary studies of this type in subjects on a rice diet have been carried out.

SOME CARDIAC AND EXTRACARDIAC EFFECTS OF DIGITALIS. *Don W. Chapman, M.D., Carroll Handley, Ph.D. (by invitation) and Russell A. Huggins, Ph.D. (by invitation). Houston, Tex.* (From the Departments of Medicine and Pharmacology, Baylor University College of Medicine.)

As a means of investigating the possible mechanism of action of digitalis, digitoxin and digoxin were used in varying concentrations from 0.1 mg. to 0.3 mg. per kg. body weight in twenty-three mongrel dogs to ascertain what relationship, if any, existed between the intravenous administration of digitalis glycosides and the serum concentration of potassium and sodium and their effect on the electrocardiographic tracings, hematocrit, the plasma volume and the extracellular fluid. Twelve additional dogs were studied in which multiple blood samples were obtained from the femoral artery by puncture and from the right atrium and coronary sinus by catheters and the serum potassium levels ascertained.

In small doses relatively little change occurred in the electrocardiograms or in the aforementioned determinations but in larger doses a roughly qualitative relationship between the increasing height of the serum potassium and the electrocardiographic alterations existed, as seen in potassium intoxication such as increase in amplitude of the T wave, decreased size of the R wave, increased amplitude of S wave, auricular arrest in some, premature ventricular contractions, intraventricular block or auriculoventricular dissociation with interference. However, in some instances no direct correlation was noted with the height of the serum potassium. Approximately the same levels of serum potassium were obtained from the heart muscle as from the skeletal muscle, indicating perhaps that there is no greater release of potassium from the heart. Marked increases in the hemato-

crit readings, a lowering of the plasma volume and in rare instances an increase in the extracellular fluid were observed when there had been marked changes in the electrocardiographic tracings.

TOXICITY OF RADIOPHOSPHORUS. *W. E. Cornatzer, M.D., George T. Harrell, Jr., M.D., David Cayer, M.D. and (by invitation) Camillo Artom, M.D. Winston-Salem, N.C.* (From the Departments of Biochemistry and Internal Medicine, Bowman Gray School of Medicine.)

The present investigation is a part of a more extended study concerning the toxicity of radiophosphorus and the factors which modify it. In the first series of experiments mice were maintained on experimental diets in which the protein and fat content were varied. After five days the mice were injected with a single dose of radiophosphorus (6 microcuries per gm. of body weight) and kept under observation for three more weeks. The per cent survival and the average survival time were greatest in mice on a low fat, low protein diet and decreased distinctly when either the protein or the fat level or both were increased (from 10 to 25 per cent protein and from 5 to 32 per cent fat). These findings may perhaps be related to an increased metabolism due to the specific dynamic action of dietary components, especially proteins, and therefore would be in accord with the concept that the susceptibility to radiation parallels the rate of cell metabolism. Young and growing tissues are known to be more sensitive to radiation and the effects of radiation seem to be more severe in animals exposed to cold or given thyroxine (both conditions causing an increase in the general metabolism).

Another series of experiments were made on mice on a low protein, high fat diet either unsupplemented or supplemented with choline. Some groups of mice also received injections of CCl_4 two to three days before the administration of radiophosphorus. There was very little difference in the per cent mortality or survival time of the various groups. One might conclude that under the conditions of our experiments neither the fatty infiltration of the liver induced by a deficiency in lipotropic factors nor the partial damage of the liver by CCl_4 modified appreciably the toxic effects of the radiophosphorus.

STEREOSCOPIC METHOD FOR OBTAINING SPATIAL VECTORCARDIOGRAM. *J. A. Cronvich, M.D., J. A. Abildskov, M.D., C. E. Jackson, M.D. (by invitation) and George E. Burch, M.D. New Orleans, La.* (From the School of Engineering, Tulane University, Department of Medicine, Tulane University School of Medicine and Charity Hospital of Louisiana.)

As reported previously the configuration of the spatial vectorcardiogram can be determined by constructing a wire model from images of projections of the vectorcardiogram onto frontal and sagittal planes. The time and labor involved in this construction is considerable. Recently, by means of a simple network of resistors between electrodes on the right arm, left arm, left leg and back, images have been obtained of projections of the vectorcardiogram onto two planes, one turned slightly to the right and the other slightly to the left of the frontal plane. By viewing these images stereoscopically the spatial characteristics of the vectorcardiogram are made evident. This method for deriving the images is admittedly approximate but the agreement between the stereoscopic image and the wire model has been satisfactory. It is hoped that this method will give impetus to the study of spatial vectorcardiography.

EXPERIMENTAL STUDY OF ACTH IN INDUCED ASTHMA. *John J. Curry, M.D., and (by invitation) Richard J. Roche, M.D., Paul D. Doolin, M.D. and Laurence H. Kyle, M.D. Washington, D.C.* (From the Georgetown University Hospital and Georgetown University Medical School.)

In a group of subjects with mild bronchial asthma the injection of histamine and methacholine resulted in an evanescent asthma-like attack with a reduction in vital capacity and maximum minute ventilation. After recovery from the attack a single intramuscular injection of 50 or 100 mg. of ACTH was given. At intervals thereafter up to seven hours and then for several days repeat injections of histamine and methacholine were administered. The subjective reaction to these drugs was noted and the vital capacity and maximum minute ventilation recorded. The effect of ACTH on the total eosinophile count of the blood was also followed.

The administration of ACTH under the conditions of our experiments was usually followed by an increase in vital capacity and maximum minute ventilation despite a lack of subjective relief from asthma. Significant protection against

the action of histamine and methacholine was not achieved, indicating that ACTH probably does not relieve asthma through an antihistaminic or anticholinergic action. The implications of these studies on the pathogenesis and treatment of bronchial asthma will be discussed.

ACUTE HEMODYNAMIC EFFECTS OF ADENOSINE TRIPHOSPHATE. *Dean F. Davies, M.D. (by invitation), Arthur L. Gropper, M.D. (by invitation) and Henry A. Schroeder, M.D. St. Louis, Mo.* (From the Hypertension Division, Department of Internal Medicine, Washington University School of Medicine.)

Adenosine triphosphate (ATP) has been reported to have a vasodilatory effect in the hindlimb of the anesthetized dog but there is some evidence that it depresses glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). Experiments were designed to study some of the hemodynamic effects of ATP in human beings. Seven unanesthetized patients were studied for the effect of 40 to 75 mg. of ATP on ERPF and GFR using para-aminohippurate and inulin clearances. ERPF fell an average of 20.2 per cent and GFR fell an average of 14.5 per cent during infusion of AIP. In six of the seven patients ERPF changes showed a close correlation with dosage. Recovery toward control values began immediately after the injection in four of six patients. No consistent changes were seen in intra-arterial blood pressure, pulse rate, or in ear, finger or toe plethysmographs in these or in six patients in whom no clearances were done. Respirations increased markedly in amplitude. However, blood pressures, pulse rates and pulse pressures of nembutalized hypertensive rates fell markedly during intravenous injection of 0.835 mg. of ATP and returned very slowly to previous levels.

Varying amounts of ATP injected into a brachial artery of hypertensive patients while continuous plethysmographic records were taken from each forefinger suggested that any vasodilator action of ATP on the arterioles did not block their response to sympathetic stimuli.

EFFECT OF DIET IN EXPERIMENTAL HYPERTENSION. *W. D. Davis, Jr., M.D., and (by invitation) William S. Jacobs, M.D., J. B. Callahan, M.D. and Durant Dabbs, M.D. New Orleans, La.* (From the Departments of Medicine, Tulane University of Louisiana School of Medicine and the Ochsner Clinic.)

Studies of the influence of diet on the blood pressure in hypertensive and normal dogs revealed the following: (1) On a high protein, high sodium diet a significant rise occurred in the blood pressure in all hypertensive animals and no increase in pressure in the normotensive control animal. (2) On a high protein, low sodium diet no significant change in the blood pressure occurred in either hypertensive or control animals. (3) On a low protein, high sodium diet no significant blood pressure response occurred in any of the hypertensive dogs tested. (4) On a low protein, low sodium diet a significant blood pressure fall was noted in all hypertensive animals and no significant change in the pressure of the normotensive control animal. In all instances the blood pressure determinations were made by direct femoral arterial puncture and the use of a mercury manometer. Hypertension was induced either by use of perirenal silk wrappings or by application of Goldblatt clamps. A total of six dogs was used; five were hypertensive, three having been hypertensive for over two years. One normotensive dog served as a control. Experimental periods on each of the diets ranged between four and eighteen weeks. A return to a previously tested diet produced results similar to the initial test period in all cases. Periods using a regular house diet either maintained or returned the pressure to pre-experimental blood levels. Lonalac, a concentrated protein, low in sodium content (Mead Johnson and Company) served as the high protein dietary constituent. Rice was used in the low protein diet. Ten grams of salt were added daily to the Lonalac and rice diet to insure a high sodium content. The caloric value of all diets was sufficient to maintain weight. Some weight loss occurred when the full diet was not taken and this was especially true on the low protein, low sodium diet.

On the average the high protein, high sodium diet produced a 25 mm. to 35 mm. blood pressure rise over pre-experimental levels in the hypertensive dogs. In these same animals a low protein, low sodium diet produced a 25 mm. to 35 mm. fall in blood pressure under pre-experimental levels.

RESPONSE OF PULMONARY ARTERIAL PRESSURE TO RAPID INFUSION OF PHYSIOLOGIC SALINE SOLUTION. *Joseph T. Doyle, M.D., Joseph S. Wilson, M.D. (by invitation) and*

James V. Warren, M.D. Atlanta, Ga. (From the Departments of Physiology and Medicine, Emory University School of Medicine.)

In the course of a study of the mechanisms of pulmonary congestion and edema 1 L. of physiologic saline solution was given intravenously in ten minutes to fourteen adult males without cardiac abnormalities. In every case the mean pulmonary arterial pressure rose by 50 per cent to 260 per cent above the control level. Both systolic and diastolic pressures participated in this rise and the pulse rate remained constant. In two other instances there was a similar response to the transfusion of whole blood. In about one-half the cases the cardiac output was unaffected by rapid infusion while in the rest there was a significant increase but this could not be correlated with the magnitude of rise in pulmonary arterial pressure. The blood volume increased as indicated by an average fall in hematocrit reading of 14 per cent. The pulmonary blood volume determined by the dye method of Hamilton as modified by Ebert showed no consistent change in eight cases.

There is therefore no evidence from this study that either increased cardiac output or increased pulmonary blood volume explains the observed rise of pulmonary arterial pressure. Pulmonary vasoconstriction or increased pulmonary venous pressure remain as possible explanations of the elevated pulmonary arterial pressure.

INFECTIONS WITH THE COXSACKIE VIRUS OBSERVED DURING AN EPIDEMIC OF POLIOMYELITIS IN TEXAS IN 1949. *Thomas W. Farmer, M.D. (by invitation), G. P. Manire, M.D. (by invitation) and S. Edward Sulkin, M.D. Dallas, Tex.* (From the Departments of Bacteriology and Neurology, Southwestern Medical School of the University of Texas.)

The variable clinical patterns presented by patients observed at the Parkland City-County Hospital in Dallas during the 1949 outbreak of poliomyelitis suggested multiple etiologic agents. Consequently, virological studies were undertaken on materials from sixty patients to determine whether agents other than poliomyelitis were present. A virus of the Coxsackie family, producing paralysis and myositis in infant mice, was isolated from two patients with paralytic infections. Both patients showed residual weakness four months after onset of illness. The strains isolated appear to be identical and are immunologically similar to strains isolated by Melnick and associates from flies trapped in the Rio Grande Valley in 1947 and from sewage collected in North Carolina in 1948. Human infections with this serologic type of Coxsackie virus have not been previously reported. Analyses of these strains, together with agents provided by Dalldorf and by Melnick, indicate that three or more serologic types exist within the Coxsackie family.

Neutralizing antibodies have been demonstrated in the sera from the two patients from whom virus was recovered. A significant rise in neutralizing antibody titer was also demonstrated in the sera of a non-paralytic patient who had been in close contact with one of the aforementioned patients. Serologic studies on specimens from other patients and from presumably normal persons are presented.

RELATION OF KIDNEY TO ACUTE PRESSOR ACTION OF DESOXYCORTICOSTERONE. *Melvin. L. Goldman, M.D., Joseph P. Kriss, M.D. (by invitation), Palmer H. Futcher, M.D. (by invitation), Henry A. Schroeder and Dean F. Davies, M.D. (by invitation). St. Louis, Mo.* (From the Hypertension Division, Department of Internal Medicine, Washington University School of Medicine.)

It has been shown that hypertensive patients have an immediate pressor response after the intravenous injection of 5 mg. of desoxycorticosterone acetate (DCA) whereas normotensive subjects do not. The pressor effect could neither be attributed to an increase in cardiac output, to retention of the sodium ion with the increase in blood volume nor to local peripheral vasoconstriction in distal parts. On the possibility that DCA (or the glucoside, DCG) might cause renal vasoconstriction and initiate the action of a renal pressor mechanism, the role of the kidneys in this response was investigated by measurement of the clearances of sodium p-aminohippurate, mannitol and chloride in human subjects and by bilateral nephrectomy in dogs. Six hypertensives, four normotensives and two patients with Cushing's syndrome were studied. In most instances the dietary intake of salt was 5 to 8 gm. per day. There was no consistent correlation except in the two patients with Cushing's syndrome whose filtration fractions

increased slightly. The ratio $M\frac{U}{P}/CL\frac{U}{P}$ decreased only in normals.

The effect of intravenous DCA or DCG upon the blood pressure before and after bilateral nephrectomy in ten mongrel dogs was measured by a Hamilton manometer. When a pressor response to DCA occurred, as it did in only five, removal of the kidneys did not abolish the response immediately but twenty-four hours and forty-eight hours later. These experiments suggest that the immediate pressor response to DCA is not mediated through the kidneys in human beings or in dogs. A direct action upon the smooth muscle of blood vessels or upon the heart remains to be proven.

EFFECTS OF VITAMIN B_{12}, GIVEN ORALLY AND PARENTERALLY, AND OF A CONCENTRATION OF "INTRINSIC FACTOR IN MACROCYTIC ANEMIAS." *Grace A. Goldsmith, M.D. New Orleans, La.* (From the Department of Medicine, Tulane University School of Medicine.)

Twenty-two patients with macrocytic anemia have been treated with oral or parenteral vitamin B_{12}, alone or combined with folic acid or a concentrate of "intrinsic factor." Complete hematologic regeneration occurred when vitamin B_{12} was given parenterally in doses as small as 0.8 μg. daily (average) in pernicious anemia and 0.4 μg. daily in sprue. Maximum rate of regeneration was attained with 3 to 4 μg. daily. The daily maintenance requirement varied from 0.3 to more than 1 μg. in pernicious anemia.

Oral vitamin B_{12}, 5 to 10 μg daily, was without effect in four patients (two pernicious anemia, two nutritional macrocytic anemia, N.M.A.) but stimulated hematopoiesis in two patients with sprue, being as effective as parenteral vitamin B_{12} in one instance.

An intestinal extract containing "intrinsic factor" failed to increase the hematologic effect of oral vitamin B_{12} which was ineffective alone in a patient with N.M.A. This potentiation has been shown in pernicious anemia (Bethell) and may assist in the differentiation of macrocytic anemias.

Combined therapy with folic acid and vitamin B_{12} led to hematologic improvement in three patients with sprue or N.M.A. receiving folic acid and in one patient with pernicious anemia receiving vitamin B_{12}.

PROGESTERONE THERAPY OF ADVANCED MAMMARY CANCER. *Douglas Gordon, M.D. (by invitation), Albert Segaloff, M.D., J. V. Schlosser, M.D. (by invitation), B. N. Horwitt, (by invitation) and P. J. Murison, M. D. (by invitation) New Orleans, La.* (From the Department of Medicine, Tulane University of Louisiana, Alton Ochsner Medical Foundation and Charity Hospital of Louisiana.)

Intramuscular injections of progesterone in aqueous suspension were used for the therapy of advanced cases of mammary carcinoma. Sixteen patients have been treated, thirteen of them for two months or longer. Of the thirteen patients, three have been stationary, nine have progressed and one showed first a mixed picture of progression and regression and then only progression. In none of the patients was there good objective evidence of regression of the neoplasm. All of the patients had local reactions from the medication, in a few instances leading to the formation of sterile abscesses.

Urinary hormonal excretions were measured in many of the patients before and during therapy. We have noted no changes in 17-ketosteroid, biologic cortin or gonadotrophin as a result of the therapy.

EVALUATION OF PERIPHERAL CIRCULATION IN MAN. *Harold D. Green, M.D., and (by invitation) C. F. McFall, M.D., R. Pollitzer, M.D. and M. Moore, M.D. Winston-Salem, N.C.* (From the Departments of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest.)

Cutaneous circulation was estimated by recording the skin temperature with the subjects' extremities exposed in a room maintained at 20°C. plus 1°. Intramuscular flow was estimated by recording the rate of uptake of 5μ curies of Na^{24}, injected 2 cm. intramuscularly, by a Geiger counter placed over the injection site. Na^{24} was reinjected when the counting rate became too low.

After cooling for one hour the torso was warmed and one hour later, while continuing the body warming, TEAC 10 to 20 mg./kg., or priscoline 1.5 to 2.0 mg./kg. were injected over thirty minutes.

The temperatures during the cooling period average 21°C. in normals and in patients with peripheral vascular disease (PVD) estimated

to be equivalent to 0.4 ml./min./100 cm.^{2}. Maximum temperatures during combined effect of the drug plus body warming in all twenty normals averaged 34°C. (equivalent to 9 ml./min./100 cm^2). Patients with PVD had maximum temperatures between 21° and 31°C.

Muscle flow was expressed in terms of the time for half the injected Na^{24} to be removed by the circulation. Twelve normal subjects during body warming averaged nineteen minutes (range eleven to thirty-one minutes); during TEAC or priscoline injection the half time averaged twenty-four minutes (range eleven to forty-two minutes). This increase is probably not significant. Three patients with hypertension had control half times averaging seventeen minutes and three patients with PVD averaged thirty-three minutes; neither were significantly changed by the vasodilator drugs.

In conclusion blood flow in the toes of normal subjects may increase twentyfold but muscle flow appears to be insignificantly affected by vasodilator procedures. Early obliterative vascular disease is probably more readily detected with cutaneous than with muscle flow measurements.

ETAMON STUDIES IN BRONCHIAL AND CARDIAC ASTHMA. *L. Gregory, M.D., M. Damiani, M.D. and A. Ruskin, M.D. (introduced by Raymond Gregory, M.D. Galveston, Tex.* (From the Department of Internal Medicine, University of Texas Medical Branch.)

In view of reports that tetraethylammonium had deleterious effects in bronchial asthma and favorable results in left ventricular failure, these two groups of patients were studied as to comparative effects of etamon, epinephrine and aminophyllin, particularly in status asthmaticus. In some cases of bronchial asthma attacks were produced with histamine and the effect of these drugs then noted. Vital capacity measurements were relied on primarily for quantitative evaluation of the effects.

In contrast to previous reports etamon was successful in relieving status asthmaticus, with evaluation of vital capacity, in one-half the cases. The effects frequently paralleled those of adrenalin and aminophyllin and those following preliminary histamine administration when the asthma was mild. In heart failure, on the other hand, the improvement in pulmonary signs, including vital capacity, was the exception rather than the rule.

PRODUCTION OF ANESTHESIA IN MUCOUS MEMBRANES BY PROCAINE AND PHYSOSTIGMINE. *Margaret E. Grieg, Ph. D., and (by invitation) W. C. Holland, M.D. and P. E. Lindvig, M.D. Nashville, Tenn.* (From the Department of Pharmacology, Vanderbilt University School of Medicine.)

We have previously reported that the selective permeability of living cells appears to be related to the activity of the acetyl choline-cholinesterase system. This was shown in mammalian erythrocytes (*Arch. Biochem.*, 23: 370, 1949) and in the blood brain barrier of the frog (*Science*, 110: 237, 1949). It is well known that procaine is not an effective surface anesthetic as it does not appear to penetrate the mucous membrane. We have found, however, that when procaine and physostigmine were together instilled into the eye (rabbit or dog), the corneal reflex was rapidly abolished (two to three minutes) and good anesthesia was produced. Physostigmine alone had no such effect. In a series of nineteen rabbits and eleven dogs it was found that the effects of physostigmine and procaine together were significantly different from those of procaine alone.

TRIAL OF BANTHINE FOR PEPTIC ULCER. *Keith S. Grimson, M.D., and (by invitation) C. Keith Lyons, M.D. Durham, N.C.* (From the Department of Surgery, Duke University School of Medicine.)

Seventy patients with peptic ulcer have been tested during the last eight months and then treated using banthine 200 to 600 mg. daily. With few exceptions ulcers were located in the duodenum. Each had roentgenologic evidence of deformity and in many evidence of crater. A majority of the patients because of pain, repeated hemorrhages or obstruction met conventional indications for surgery. Three with obstruction caused by scar tissue subsequently required gastroenterostomy with vagotomy. The remainder are continuing use of banthine without other medication and usually without dietary restriction.

Roentgenograms obtained before treatment in each patient and at intervals afterward have evidenced improvement with healing of craters when present. With few exceptions pain was relieved by the first dose of banthine and with continuing treatment discomfort has not recurred. Three patients, however, described increase of pain with 100 mg. doses orally. Occasionally

symptoms of gastric retention developed during the first several days of treatment and then subsided. Usual therapeutic dose has been 100 mg. every six hours emphasizing one dose during the middle of the night. Maintenance dose instituted after several weeks of treatment has been 50 mg. every six hours. Clinical results at present are remarkably encouraging.

EFFECTS OF PYROGEN-INDUCED FEVER ON CEREBRAL FUNCTION IN NEUROSYPHILIS. *Albert Heyman, M.D., and (by invitation) John L. Patterson, Jr., M.D. and Fenwick T. Nichols, Jr., M.D. Atlanta, Ga.* (From the Departments of Medicine and Physiology, Emory University School of Medicine, and Grady Memorial Hospital.)

The effects of pyrogen-induced fever on the cerebral blood flow and oxygen consumption were determined, utilizing the nitrous oxide technic, in thirteen patients with asymptomatic neurosyphilis and fourteen patients with dementia paralytica. The mean elevation of temperature in both groups of patients was 3.9°F. Each patient was studied at normal body temperature and in the flush phase of fever.

In the patients with asymptomatic neurosyphilis the mean cerebral blood flow and oxygen consumption were normal in the afebrile state and showed only minimal changes during fever. In the patients with dementia paralytica the mean cerebral blood flow and oxygen consumption, which were abnormally low in the afebrile state, increased by 30 per cent and 23 per cent, respectively, during fever.

It is concluded that during induced fever the brain in asymptomatic neurosyphilis does not share in the increases in cardiac output and total oxygen consumption which are known to occur. By contrast the brain in dementia paralytica shares to some extent in the increases in both of these functions. The beneficial effect of fever therapy in dementia paralytica may have some relation to these findings. It is tentatively assumed that the normal brain has a response to fever similar to that of the asymptomatic patients.

VITAMIN B_{12} AND PTEROYLGLUTAMIC ACID DEFICIENCY IN MACROCYTIC ANEMIAS. *Ulfar Jonsson, M.D., (by invitation) and R. Wayne Rundles, M.D. Durham, N.C.* (From the Department of Medicine, Duke University School of Medicine.)

A series of observations suggests that the basic deficiency in pernicious anemia is different from that in other macrocytic anemias associated with megaloblastic erythropoiesis.

In patients with pernicious anemia followed for as long as one and one-half years vitamin B_{12} has duplicated in all respects the therapeutic results of liver extract. Incomplete responses and relapses such as occur with pteroylglutamic acid therapy have not occurred. Intrinsic factor has been shown to increase the absorption of B_{12} (extrinsic factor) in pernicious anemia. While pernicious anemia ordinarily develops only when intrinsic factor is absent, a patient was studied who had a macrocytic anemia, megaloblastic bone marrow, atrophy of lingual papillas and severe neurologic disease characteristic of pernicious anemia but who had abundant free HCl in the gastric contents. Assay showed intrinsic factor to be present. All manifestations of the deficiency responded to B_{12} injections.

Of six infants with megaloblastic anemia one failed definitely to respond to B_{12} but responded promptly to pteroylglutamic acid.

In an adult woman who used alcohol heavily nutritional macrocytic anemia developed that failed to respond in a month's time to large amounts of liver extract intramuscularly. She responded rapidly to pteroylglutamic acid by mouth. An episode of nutritional macrocytic anemia developed in an alcoholic male for the third time while he was being given ordinarily adequate amounts of B_{12} and liver extract intramuscularly. He responded to pteroylglutamic acid by mouth.

These observations are consistent with the hypothesis that pernicious anemia is due to B_{12} deficiency, however it arises, and other macrocytic anemias are due to pteroylglutamic acid deficiency. Each compound may serve as a partial therapeutic substitute for the other.

ADRENAL INSUFFICIENCY SIMULATING CARDIAC DISEASE. *Laurence H. Kyle, M.D. and Catharine Q. Knop, M.D. (introduced by Harold Jeghers, M.D.). Washington, D.C.* (From the Departments of Medicine and Pediatrics, Georgetown University School of Medicine and the Georgetown University Hospital.)

All three children in one family have shown a similar clinical picture resulting in death. The first child, a ten-day old boy with "hypospadius and undescended testes," began to vomit, became severely dehydrated and died. The clinical

diagnosis was intestinal obstruction. The second child, a boy, showed similar symptoms at the same age. Because of marked bradycardia, a loud apical systolic murmur and electrocardiographic evidence of A-V and intraventicular block, a diagnosis of congenital septal defect was made. Autopsy revealed no cardiac lesion. The third boy was similarly affected. Bradycardia was prominent and the electrocardiogram was interpreted as showing auricular fibrillation and bundle block; after the administration of fluids the heart rate and electrocardiogram became normal. There was elevation of serum potassium and NPN with depression of sodium and chloride. Administration of desoxycorticosterone resulted in marked clinical improvement and change of serum electrolytes to normal. Urinary 17-ketosteroids were elevated and 11-oxysteroids absent.

These children are believed to have had congenital adrenal hypoplasia, a condition which frequently leads to adrenal failure. It is believed that this syndrome is frequently overlooked, especially in boys, clinical features being attributed to intestinal obstruction. Simulation of cardiac disease has not previously been noted.

CONGENITAL IDIOPATHIC HYPOPROTHROMBINEMIA. *Jessica H. Lewis, M.D. and John H. Ferguson, M.D. Chapel Hill, N.C.* (From the Department of Physiology, University of North Carolina Medical School.)

Two patients, unrelated and of different sexes, who had suffered from hemorrhagic manifestations since infancy were found to have markedly prolonged prothrombin times (Quick), indicating "prothrombin concentrations" of 1 to 2 per cent. In one patient, R. S., the coagulation time (37°C.) was prolonged to eighty-eight minutes (glass) and three and a half hours (silicone), while in the other patient, M. T., the coagulation time was only slightly prolonged to thirteen minutes (glass) and forty-five minutes (silicone). Results of other hemostatic tests: bleeding time, platelet count, tourniquet test, clot retraction, plasma fibrinogen, serum antithrombin, ability to normalize hemophilic coagulation, absence of anticoagulant or progressive antithromboplastin were normal in both patients. Special studies were made of these "prothrombin" deficiencies by mixing the plasmas with aged plasma, dicumarol® plasma, $BaSO_4$ plasma and by adding Seeger's bovine prothrombin and Ac-globulin. It was concluded that no deficiency of Quick's labile factor existed in either case but that prothrombin, probably Quick type B, was markedly diminished. Although both patients had received frequent small doses of synthetic water soluble vitamin K derivatives without apparent benefit, therapeutic trials of 1,000 mg. of synkayvite and 1,000 mg. of vitamin K-1 oxide intravenously were made in one patient, M. T., and no shortening of prothrombin time was observed.

EFFECTS OF BLEEDING IN DIFFERENT POSTURES ON SODIUM EXCRETION AND GLOMERULAR FILTRATION. *Thomas Lombardo, M.D. and William Viar, M.D. (introduced by T. R. Harrison, M.D.). Dallas, Tex.* (From the Department of Internal Medicine, Southwestern Medical College.)

Minimal bleeding (200 cc.) in the sitting posture caused definite decline in sodium excretion without change in glomerular filtration. Bleeding larger volumes in the recumbent or Trendelenburg positions caused temporary decline in glomerular filtration in most of the subjects. The decline in sodium excretion was maximal two to three hours after the filtration rate had returned to normal. The alterations in sodium excretion are therefore to be ascribed to changes in tubular activity.

EFFECTS OF BANTHINE ON GASTRIC SECRETIONS AND GASTROINTESTINAL MOTILITY IN MAN. *C. Keith Lyons, M.D. (by invitation) and Keith S. Grimson, M.D. Durham, N.C.* (From the Department of Surgery, Duke University School of Medicine.)

Banthine, a quaternary ammonium compound, beta-diethylaminoethyl xanthene 9-carboxylate methobromide, has been tested in 120 patients in studying effects of 100 or 200 mg. by intravenous, intramuscular or oral administration and comparing effects with those of atropine or dibutoline. As judged by gastrometric balloon methods and by roentgenographic study, banthine given intramuscularly eliminated or effected a marked reduction of peristalsis lasting several hours. Atropine intravenously or intramuscularly produced a less consistent reduction lasting around half an hour but dibutoline effected for an hour as complete a cessation of activity as banthine. Banthine given orally produced marked reduction or elimination of peristalsis lasting several hours; whereas, atropine orally effected only slight temporary changes and dibutoline orally was not effective.

Effect on gastric secretions of each drug by each route of administration was also determined. Results were variable. Banthine effected more frequent, more pronounced and longer lasting reduction of volume and acidity than atropine or dibutoline (i.v. or i.m.). With oral administration banthine was effective, atropine had little effect and dibutoline no effect. During the period of reduction of free acid an hour or more after banthine, 100 mg. or with better consistency 200 mg., insulin hypoglycemia failed to produce high acid in some patients, elevation occurring in others.

ACETYLATION OF P-AMINO COMPOUNDS BY THE RABBIT. *M. F. Mason, M.D., Gus Casten, M.D. (by invitation) and Allan Lindsay, M.D. (by invitation). Dallas, Tex.* (From the Parkland Hospital and the Southwestern Medical School of the University of Texas.)

Following intraperitoneal injection of 200 to 500 mg. of certain p-amino compounds in the rabbit, acetylation by the liver attains a maximum rate which is then essentially independent of the plasma concentration of the p-amino compound achieved. The acetylation rate is calculated by measuring the amount of acetylamino derivative appearing in the urine and accumulating in the body per unit time. Calculations based on the ultimate plasma concentrations obtained after similar injections into nephrectomized rabbits indicate the fluid volume in which the drug is distributed. Preliminary estimates of the maximum rates of formation of acetylsulfanilamide, acetylaminobenzoate and acetyl p-amino hippurate have been made.

EXCHANGE OF ALBUMIN BETWEEN PLASMA AND LYMPH. *H. S. Mayerson, M.D. (by invitation) Kalman Wasserman, M.D. New Orleans, La.* (From the Department of Physiology, Tulane University School of Medicine.)

Human serum albumin tagged with radioactive I, (I-131), has been injected intravenously in dogs and its appearance followed in thoracic duct lymph for periods as long as thirty-six hours. The iodo-albumin leaves the plasma exponentially at a very slow rate and appears in the lymph in measurable count in from ten to twenty minutes after the injection. Analysis of curves drawn from specific activity determinations indicate that the uptake of iodo-albumin in thoracic duct lymph is quite rapid at first then shows a slower phase which, in turn, is followed by a still slower uptake until equilibrium is reached in about fourteen hours (as determined by extrapolation). Thereafter the albumin seems to disappear from both tissues at approximately the same rate. Curves drawn from the results of experiments in which the blue dye, T-1824, was injected simultaneously with the iodo-albumin reveal differences, particularly in the last phase of lymph uptake. The results to date allow certain tentative conclusions with respect to the permeability of blood and lymph capillaries as well as to the volume of available extracellular fluid.

ELECTROKYMOGRAPHIC TRACING OF LEFT AURICULAR PULSATIONS. *J. B. McKinnon, M.D. (by invitation) and Ben Friedman, M.D. Dallas, Tex.* (From the Veterans Administration Hospital, McKinney, Texas and the Department of Medicine, Southwestern Medical College.)

Luisada and his co-workers have described a characteristic change in the electrokymographic tracing taken over the left auricle in mitral valve disease. Essentially it consists of increased auricular filling with the onset of ventricular systole. We have confirmed this finding. It is believed to be due to actual regurgitation of blood from ventricle to auricle and not to positional change of the heart. It did not appear in patients with right ventricular hypertrophy due to cor pulmonale nor in subjects with the Austin Flint murmur associated with aortic insufficiency. The typical curve has been observed in persons with mitral stenosis without an audible systolic murmur. While regurgitation may be present with mitral stenosis in the absence of a systolic murmur, instances of pure mitral stenosis without electrokymographic or ausculatatory evidence of insufficiency have been noted. In far advanced mitral stenosis with severe congestive failure electrokymographic evidence of regurgitation tends to be minimal.

The characteristic electrokymographic tracing was not found in persons with loud apical systolic murmurs, associated with large hearts due to non-valvular heart disease. These observations cast doubt on the concept of functional mitral insufficiency in relation to cardiac enlargement.

EFFICACY OF QUINIDINE IN PREVENTING VENTRICULAR FIBRILLATION INDUCED BY TEMPORARY CORONARY OCCLUSION IN DOGS. *George R. Meneely, M.D., and (by*

invitation) *Sam E. Stephenson, M.D. and Otto M. Kochtitsky, M.D. Nashville, Tenn.* (From the Research Laboratory of Thayer Veterans Administration Hospital and the Department of Medicine of Vanderbilt University School of Medicine.)

We have developed a technic for inducing ventricular fibrillation in a high proportion of dogs by a mechanism which may have a counterpart in human coronary artery disease. The entire anterior descending branch of the left coronary artery was occluded for thirty minutes and then allowed to reopen. Such a procedure provides a suitable method for assay of antifibrillatory agents because ventricular fibrillation occurred in 75 per cent of twenty-eight untreated dogs.

When 15 mg./kg. of quinidine were administered slowly intravenously during the occlusion in fifteen more dogs, fibrillation after release of the artery developed in only four or 27 per cent, a large and significant improvement over the usual 75 per cent in controls. The dose of 15 mg./kg. was chosen arbitrarily and is almost certainly not optimal in the light of other work by France and Kory, nevertheless, even this low dose was quite effective as seen from the aforementioned data.

A Fat Emulsion for Intravenous Nutrition in Rabbits. *H. C. Meng, M.D.* (*introduced by William J. Darby, M.D.*). *Nashville, Tenn.* (From the Department of Physiology, Vanderbilt University Medical School.)

It was previously reported from this laboratory that a fat emulsion, administered intravenously to dogs, was non-toxic and could be utilized. The present report indicates similar results in experiments with rabbits. Normal controls were observed for two weeks with blood counts and determination of hemoglobin and body weight. Twenty rabbits then received daily intravenous injections as follows: two received 5 per cent glucose, five received the emulsifying agents in 5 per cent glucose and thirteen received 10 per cent fat emulsion in 5 per cent glucose. The daily amount of fat was 1.5 gm. per kg. of body weight. In some animals injections were continued for four weeks after which the animals were sacrificed for histologic study. There had been no significant change in the blood picture and there was no noteworthy change in the microscopic picture of the liver, lung, heart, kidney or spleen. Pyrogen tests showed that emulsions which are less than two months old are pyrogen-free but older emulsions can be rendered less likely to produce fever if re-autoclaved. Furthermore, it has been found that the emulsion is not antigenic and the intravenous injection of 1.5 to 3.0 gm. of fat per kg. of body weight as 10 per cent emulsion produced no appreciable effect on arterial blood pressure. There is every indication that this emulsion could be used clinically.

Further Studies on the Existence of Renal Bypasses. *John H. Moyer, M.D. and Carroll A. Handley, M.D.* (*introduced by James A. Greene, M.D.*). *Houston, Tex.* (From the Department of Medicine and Pharmacology, Baylor University College of Medicine.)

An attempt was made to evaluate further the existence of cortical ischemia resulting from juxtamedullary shunts in the kidney. These were reported by Trueta et al. to become active functionally following sciatic nerve stimulation or the administration of adrenalin. Their conclusions were based on the concentration of dye in the juxtamedullary area of the kidney and on arterialization of blood as noted on direct observation.

In the experiments on dogs herein reported changes in renal blood flow as measured directly, systemic blood pressure, renal arteriovenous oxygen differences were determined following sciatic nerve stimulation. Renal blood flow consistently decreased (40 per cent) irrespective of systemic blood pressure which increased in about three-fourths of the animals. The mean A-V O_2 difference increased (100 per cent after two hours) significantly due to decreasing oxygen content of the blood coming from the kidney. Arterialization was never observed. Similar changes were noted following the administration of adrenalin.

An attempt was then made to increase renal blood flow by the use of thyroxin. Renal blood flow, glomerular filtration and glucose Tm were measured by indirect clearance methods. After five days of thyroxin administration all three were found to increase (40 per cent) in direct proportion. Similar observations were noted in an acute experiment using dinitrophenol to increase renal blood flow and general metabolic function. An experiment was then set up to do clearance studies, direct blood flow and renal blood oxygen content studies when renal blood

flow is increased by the use of dinitrophenol and decreased following sciatic nerve stimulation. No evidence was found to support the hypothesis of "renal bypasses."

STUDIES OF HEPATIC FUNCTION IN PATIENTS ON THE "RICE DIET." *J. D. Myers, M.D. and (by invitation) R. J. F. Murphy, M.D. Durham, N.C.* (From the Department of Medicine, Duke University School of Medicine.)

The hepatic blood flow, splanchnic oxygen consumption, net splanchnic glucose output and the bromsulfalein clearance have been estimated in a group of subjects treated with the Kempner "rice diet." The data so obtained have been compared with similar figures obtained on control individuals without significant disease and with another series of patients with arterial hypertension but not receiving the rice diet.

The hepatic blood flow and splanchnic oxygen consumption have been found normal in the subjects on the rice diet. The net splanchnic glucose production is reduced in some individuals. A decided decrease in bromsulfalein clearance makes its appearance during the first several weeks on the diet and persists thereafter for as long as three months. The BSP clearance is reduced not only in comparison to normal subjects but in comparison with the non-treated hypertensives, also. The disability in removal of BSP from the blood parallels in general the reduction in serum cholesterol which these patients characteristically show. Supplementation of the diet with cholesterol has thus far not prevented the fall in either BSP clearance or in serum cholesterol values.

A POSSIBLE MECHANISM FOR THE HYPOTENSIVE EFFECT OF THIOCYANATES. *Norman S. Olsen, M.D. (introduced by Henry A. Schroeder, M.D.) St. Louis, Mo.* (From the Hypertension Division, Department of Internal Medicine, Washington University School of Medicine.)

Thiocyanates have been used successfully in therapeutically lowering blood pressure in selected hypertensive patients. Inasmuch as little is known concerning its mechanism of action, the effect of this ion was studied *in vitro*. Two distinct actions of thiocyanate have been found in this study. The first, a general toxic effect on tissue oxidation, is probably a reflection of the untoward symptoms noted in patients. *In vitro* the toxic effect is obtained at levels of about $0.1M$ thiocyanate. It was also found that cyanide in concentrations of $0.001M$ produced the same result and a mixture of the two inhibitors results in no further action. The second action seems to be an inhibition of amino acid oxidation at a concentration of about $0.0005M$ thiocyanate, at which concentration little change is found in basal tissue oxidations. At these concentrations cyanide produces no inhibition of amino acid oxidation. Thiocyanate exerts no effect on the oxidation of added amines at these low concentrations.

It has been postulated that faulty amino acid and amine metabolism are important in the pathogenesis of essential hypertension. If pressor amines are of significance in causing or maintaining hypertension, any agent which diminishes the production of amines from this parent amino acid should produce a hypotensive effect. It is found that thiocyanate produces a hypotensive effect at concentrations five times greater than those required to inhibit amino acid metabolism.

RELATION OF PLASMA CELL GROWTH TO ABNORMAL SERUM PROTEIN COMPONENTS AND BENCE JONES PROTEINURIA IN MULTIPLE MYELOMA. *R. W. Rundles, M.D. and (by invitation) M. L. Dillon, M.D., Edith S. Dillon, M.D. and G. R. Cooper, M.D. Durham, N.C.* (From the Department of Medicine, Duke University School of Medicine.)

The relationship of plasma cell proliferation to the abnormal serum proteins and to Bence Jones proteinuria in multiple myeloma has been studied during urethane therapy. In most patients 90 to 300 gm. of urethane given in a period of six to ten weeks reduces the number of abnormal plasma cells in the bone marrow and produces morphologic changes indicative of arrested or retarded growth. Sixty electrophoretic analyses of the serum proteins were made in eleven patients followed from three to twenty-eight months. Six patients with hyperproteinemia had large peaks of abnormal protein with gamma mobility. After two to four months of therapy three of them had virtually normal patterns, only slight homogeneity remaining in the gamma globulin. In a fourth patient protein with gamma mobility was reduced about 50 per cent. In two there was no change.

One patient had a large protein increment with mobility intermediate between gamma and

beta globulin. He obtained a partial remission during the first treatment period but relapsed after three months. Urethane was then given continuously, 1,275 gm. in fourteen months. He relapsed under treatment and urethane was discontinued. The excretion of Bence Jones protein in the urine then declined from an average of 17 to 19 gm. per day to 0.25 to 0.4 gm., serum protein with the mobility of the abnormal component fell from 2.35 gm. to 1.0 gm. per 100 cc. and the hemoglobin which had fallen below 6.0 gm. per 100 cc. was again maintained at normal levels.

In one of the four patients with a virtually normal serum protein pattern a definitely abnormal peak developed during an exacerbation of his disease after being followed for seventeen months. In another patient heavy Bence Jones proteinuria was reduced 75 per cent without altering the serum protein pattern.

As plasma cell growth is inhibited by urethane in multiple myeloma, abnormal serum protein components, even those generally found not to represent Bence Jones proteinemia, are reduced or may virtually disappear. This change parallels the reduction or disappearance of Bence Jones proteinuria. During prolonged continuous administration of urethane myeloma cells may become dependent on the chemical.

CEREBRAL METABOLISM IN HYPERTHYROIDISM AND MYXEDEMA. *Peritz Scheinberg, M.D. (introduced by Eugene A. Stead, Jr., M.D.). Durham, N.C.* (From the Duke University School of Medicine.)

Cerebral blood flow and metabolism were measured by means of the nitrous oxide technic in nine subjects with hyperthyroidism and in eight subjects with myxedema. Three patients with myxedema were restudied after clinical improvement on thyroid therapy.

The subjects with hyperthyroidism showed no significant variation from normal in any of the measured cerebral metabolic functions. This is of interest in view of the 35 per cent increase in splanchnic oxygen consumption known to occur in a similar series of hyperthyroid patients.

The patients with myxedema showed reductions in cerebral blood flow (38 per cent) and oxygen consumption (27 per cent) commensurate with the fall in cardiac output and total oxygen consumption which occurs in these patients. Cerebral glucose consumption decreased in proportion to the cerebral oxygen consumption. Cerebral vascular resistance was increased almost 100 per cent. All cerebral metabolic functions returned toward normal along with clinical improvement in the three patients restudied after thyroid therapy.

These data indicate that in hyperthyroidism the brain does not share in the general increase which occurs in body metabolism and that the clinical signs of mental dysfunction so commonly observed in myxedema may be accounted for by the decreased cerebral metabolism in this disease.

PATHOGENESIS OF JARISCH-HERXHEIMER REACTION IN RABBIT SYPHILIS: PRODUCTION BY THE INJECTION OF SYPHILITIC SERUM. *Walter H. Sheldon, M.D., Albert Heyman, M.D. and (by invitation) Lilian D. Evans, M.D. Atlanta, Ga.* (From the Departments of Pathology and Medicine, (Clinic for Genitoinfectious Diseases), Emory University School of Medicine, and Grady Memorial Hospital.)

Previous studies have shown that transient acute inflammatory changes occur in human syphilitic lesions during the Herxheimer reaction. Similar histologic changes occur in syphilitic lesions of rabbits following treatment. Injections of living or dead spirochetes failed to produce these changes.

Herxheimer-like reactions occur in Spirillum minus infections in which immobilizing and lysing antibodies are present. This infection produces skin lesions in rabbits. When serum from similarly infected rabbits was given, the recipient animals showed histologic changes in the lesions resembling the changes occurring in the Herxheimer reaction of syphilis.

Twelve rabbits were inoculated intravenously and in multiple skin sites with Treponema pallidum, Nichols strain. After lesions appeared each of the six animals was given 70 cc. of serum intravenously from untreated syphilitic rabbits. The remaining animals received equal amounts of normal rabbit serum. Individual syphilomas were excised from each animal before and after injection. On histologic examination transient acute inflammatory changes were found in the syphilomas of five animals receiving syphilitic serum. No changes were observed in the six controls.

Our findings suggest a relationship of serum antibodies in syphilitic and Spirillum minus infections to the mechanism of the Herxheimer reaction. Further studies on this problem are in progress.

STUDIES ON AN HOMOLOGOUS BRAIN TISSUE ANTIGEN IN DOGS. *Lewis Thomas, M.D.,*

(*and by invitation*) *Philip Y. Paterson, M.D. and Elizabeth Smithwick, M.D. New Orleans, La.* (From the Division of Infectious Disease, Department of Medicine, Tulane University School of Medicine.)

Allergic encephalomyelitis has been produced in dogs by immunization with homologous brain extracts mixed with adjuvants. The histopathology of the disease resembles that described by others in monkeys but a longer time is required for the development of lesions and the incidence is lower. An antibody has been demonstrated in the serum of immunized dogs which reacts with an antigenic component of normal dog brain and peripheral nerve in complement fixation tests. The relationship between this antigen-antibody system and the experimental disease is not known; all dogs showing demyelination have had antibody but others without lesions have also had antibody.

The antigen is demonstrable in aqueous and lipid-solvent extracts of brain. It is present in brain and nerve tissue from other species but not in other organs. It is not demonstrable in newborn dog brain tissue. It is resistant to boiling and to treatment with 10 per cent formalin. It is a component of the acetone-soluble, unsaponifiable fraction of white matter lipids and is separable from cholesterol. Specific immunologic activity may be demonstrated with less than 1 microgram of the partially purified material. Further studies bearing on the nature of this material will be presented.

EFFECTS OF POSTURE AND OF COMPRESSION OF THE NECK ON GLOMERULAR FILTRATION. *William Viar, M.D. and Thomas Lombardo, M.D.* (*introduced by T. R. Harrison, M.D.*). *Dallas, Tex.* (From the Department of Medicine, Southwestern Medical College.)

The rise in sodium excretion produced by the recumbent position or by compression of the neck in the sitting position is not attended by significant change in glomerular filtration. Alterations in tubular activity must therefore be held responsible.

RELATIONSHIP OF DURATION OF PHASES OF CARDIAC CYCLE LENGTH AS DETERMINED IN HUMANS BY THE ELECTROKYMOGRAPH. *Kathryn Willis, M.D.* (*introduced by Tinsley R. Harrison, M.D.*). *Dallas, Tex.* (From the Department of Internal Medicine, Southwestern Medical College.)

There is a paucity of data in the literature of the duration of phases of the cardiac cycle in human subjects. Therefore, it seems worth while to present the duration of these phases and relation to cycle length as determined in normal subjects by the electrokymograph. The average values are, namely, isometric contraction 0.039 second, rapid ejection 0.104 second, total systole 0.26 second, protodiastole (time from relaxation of ventricle to closure of semilunar valve) 0.039 second, as measured from tracings of ascending aorta in thirty-two normal subjects. Diastolic phases measured from left ventricular border tracings are the following: isometric relaxation 0.104 second, rapid filling 0.135 second and diastasis 0.16 second. Ranges of these values are to be presented as well as comparison to total cardiac cycle length.

TREATMENT OF INFECTIONS DUE TO PSEUDOMONAS AERUGINOSA (BACILLUS PYOCYANEUS) WITH POLYMYXIN—REPORT OF A CASE OF MENINGITIS. *Ellard M. Yow, M.D. and E. R. Hayes, M.D.*) (*introduced by James A. Greene, M.D.*). *Minneapolis, Minn.* (From the Department of Medicine, Baylor University College of Medicine, Houston, Texas and Students' Health Service, University of Minnesota.)

Polymyxin is a polypeptide antibiotic which acts as a bactericidal agent against Aerobacter aerogenes, Sal. typhosa, K. pneumoniae, H. influenzae, and Pseudomonas aeruginosa but not against Proteus vulgaris. It is composed of five fractions having similar chemical and biologic properties but varying somewhat in toxic manifestations. Because of evidences of nephrotoxicity and neurotoxicity occurring in some patients, as well as the overlapping antibacterial spectrum of other antibiotic agents, its general use has been limited. Infections due to Pseudomonas aeruginosa, however, frequently do not respond to other available antimicrobial agents. Clinical experience thus far has revealed a consistently favorable response in patients with Pseudomonas infections treated with polymyxin. Consequently, it is believed that because of the high mortality rate associated with systemic infections due to this organism, polymyxin represents the most successful means of treatment available at the present time.

A case of meningitis due to Pseudomonas aeruginosa occurring in a sixteen year old girl following spinal anesthesia, failing to respond to sulfonamide, penicillin and streptomycin therapy but responding to the intramuscular and intrathecal administration of polymyxin, is discussed.

Case Reports

Disseminated Coccidioidomycosis*

Treatment with Protoanemonin

NEAL J. CONAN, JR., M.D. *and* GEORGE A. HYMAN, M.D.
New York, New York

GENERALIZED, or disseminated, coccidioidomycosis was first described as a disease entity in man by Wernicke[1] in Argentina in 1892. Four years later Rixford and Gilchrist[2] reported two cases in North America. It was, however, not until forty years later[3,4] that coccidioidomycosis became well recognized as endemic to certain arid areas of California, Arizona, New Mexico and West Texas.[5–8]

Our present day understanding of this disease has evolved to a considerable extent on the basis of three types of study: (1) by virtue of further documentation of the characteristics of the organism[9] as well as the pathogenesis of the infection;[3,4,6,10,11,32] (2) through recognition of the disease by the large scale employment of the skin sensitization reaction,[12,13] particularly by Jacobson;[14] (3) through the practical utilization of the complement-fixation and precipitin tests in diagnosis as a result of the pioneering efforts of Smith[15] and Cooke.[16]

Although an extensive animal reservoir has been found in endemic areas, in wild rodents, dogs, sheep, cattle and various anthropoids, transmission from animal to man or man to man has not been described.[17,18] Ordinarily the highly infectious chlamydospores present in the dust of endemic areas are inhaled and thereby may initiate the disease. According to Smith[9] "the only significant portal of entry is the respiratory. Occasional infections have been credited to abrasions.[19,20] Infection by ingestion does not occur." There appears to be general agreement with this viewpoint[21,22] and other portals of entry have only rarely been incriminated. Two cases of rather widespread involvement of the female internal genital organs have been described to date[24,25] but there is no reference in the literature to the uterus as a possible primary focus.

The present report concerns a patient with disseminated coccidioidomycosis in whom the portal of entry appears to have been the postpartum uterus.

CASE REPORT

A thirty year old, white Jewish housewife was admitted to the Presbyterian Hospital in January, 1946, because of a two-year febrile illness which, in the preceding six weeks, had become rapidly progressive. Her son aged six and her daughter aged two and one-half, the issues of her only pregnancies, as well as her husband, were in good health. They had no evidence of coccidioidomycosis then or at the time of the present writing. Positive features of the past history were uncomplicated scarlet fever as a child, seasonal hay fever of twelve years' duration, ascariasis successfully treated, and an attack of "pyelitis" marked by hematuria. The last two diseases occurred in 1938 during a year spent in Syria and Turkey. Except for this year in the Near East she had always resided in New York City until 1941.

Between 1941 and 1945 the patient lived in Arizona and New Mexico where her husband was a physician on an Indian reservation. She was well until May, 1943, when during the seventh month of her second pregnancy there was a transient episode of uterine bleeding. Delivery occurred at term in July and was said to have been normal except that it was "dry."

* From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y.

The puerperium was complicated by failure of the uterus to involute but there were no signs of infection as manifested by fever or leukocytosis. Because involution did not occur within two months a dilatation and curettage was performed in an Arizona hospital on September 17, 1943, following which the uterus involuted and normal menses ensued. The histopathologic diagnosis made upon the curettings was tuberculosis of the endometrium. An x-ray of the chest was reported as normal. At no time prior to or during puerperium were there symptoms of a respiratory infection nor were there skin lesions. For the next three and a half months the patient enjoyed good health.

Between January, 1944, and January, 1945, the patient experienced intermittent episodes of malaise, chilliness and fever to 100°–101°F. These lasted from two to seven days and occurred every one or two months. During this year skin tests with coccidioidin and tuberculin were negative and additional x-rays of the chest revealed no abnormalities other than what was interpreted as healed, calcified, childhood tuberculous lesions in the hilar areas. In August, 1944, an erythematous, macular lesion appeared in the right nasolabial fold. This did not progress but persisted for one year. Three months later a 39 per cent eosinophilia and mild anemia were discovered. From January, 1945, to August, 1945, the patient was asymptomatic. In July, 1945, the nasolabial skin lesion was biopsied and diagnosed as lupus vulgaris. It was then treated with Grenz rays and subsequently disappeared.

The patient returned to New York City in September, 1945, when the low-grade fever recurred and her menses became irregular and scanty. Now for the first time parenchymal pulmonary lesions were demonstrated by x-ray. These were described as "soft areas located in the mid-lung fields and at the right base" with disappearance within two weeks.

Early in December, 1945, the patient's fever rose to daily peaks of 103°–105°F., associated with chills, sweats, substernal discomfort, lassitude and weakness, with a 12 pound weight loss in a month. Four weeks later dyspnea, cyanosis and a paroxysmal cough supervened. There was no orthopnea or edema. During the last four of these six weeks of severe illness the patient was under treatment at another hospital in New York. Positive findings on physical examination included rales at the right lung base and enlargement of the spleen to 3 cm. below the costal margin. Further work-up revealed anemia, eosinophilia, hyperglobulinemia and pulmonary infiltrations. Trichinella precipitin test was positive 1:1200. Attempts to identify an etiologic agent in blood, sputum and feces, as well as by means of serologic and skin tests were unsuccessful. At various times during the two-year illness the patient received courses of quinine, sulfonamides, penicillin, gold and aspirin without effect. She was transferred to Presbyterian Hospital on January 19, 1946.

On admission, physical examination revealed an acutely and chronically ill thirty year old female who was feverish, cyanotic, dyspneic and orthopneic. Her temperature was 105°F., heart rate 130 per minute, respiratory rate 35 per minute and blood pressure 105/60 mm. of mercury. There were no skin eruptions and the lesion earlier described in the right nasolabial fold could barely be detected. The eyes, ears, nose, throat and neck were normal except for the ocular fundi in which there were bilateral striated hemorrhages. The right fundus showed in addition perivascular sheathing and two large areas of exudate. Bilateral small, firm, tender axillary and epitrochlear nodes were present. The spine, thorax and breasts were normal. The right lung base was flat to percussion with absent breath and voice sounds, superior to which could be heard scattered rales and bronchi. The heart was enlarged, with a sinus tachycardia and an apical gallop rhythm present, but no murmurs were heard. The liver was at the right costal margin and a firm tender spleen was palpated 5 cm. below the left costal margin. Pelvic and rectal examinations were normal, except for a small, tender, retroverted uterus; no vaginal discharge was present. The nervous system and extremities showed no abnormalities.

On several blood counts the hemoglobin ranged from 10.2 to 11.0 gm., red blood cells from 3.52 to 4.25 million, white cells from 6,750 to 12,700, neutrophils 35 to 42 per cent, and eosinophils from 35 to 41 per cent. Preterminally the neutrophils rose to between 60 to 90 per cent, the eosinophils fell to between 2 and 16 per cent, with the appearance of 2 to 10 per cent myelocytes and 1 to 3 per cent myeloblasts. The sedimentation rate (Westergren) was 72 mm. in one hour. Urinalysis revealed a specific gravity of 1.020 with 1 to 2 plus albuminuria, occasional white cells and

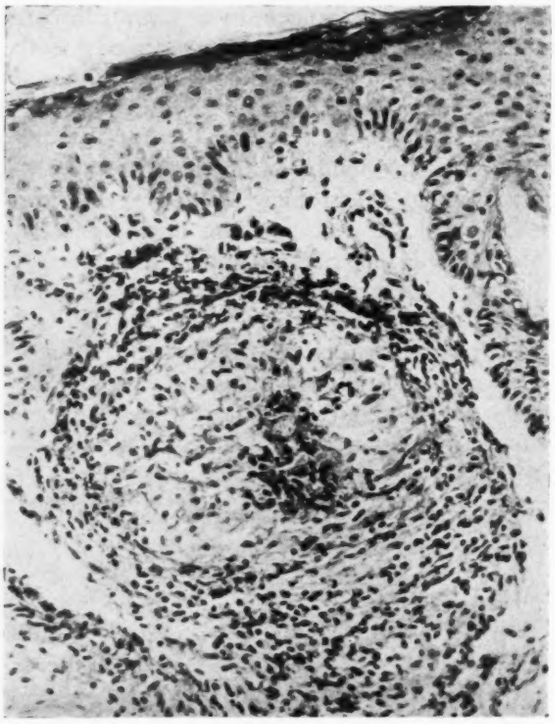


FIG. 1. Biopsy specimen of the papular lesion of the right nasolabial fold, taken in July, 1945, which demonstrates the doubly refractile spherule of Coccidioides immitis at the center of a subcutaneous granulomatous lesion.

on one examination a few red blood cells. The stool guaiac test was negative. Chemical determinations upon the blood serum gave the following results: albumin 4.0 gm. per cent, globulin 3.4 gm. per cent, euglobulin 0.6 gm. per cent, urea nitrogen 7 mg. per cent, a faint trace of bilirubin, alkaline phosphatase 12.5 Bodansky units, and cholesterol 196 mg. per cent. The cephalin-cholesterol flocculation test was negative. Electrocardiograms showed only sinus tachycardia, later digitalis effect. X-ray film of the chest revealed a normal heart shadow, calcifications in the hilar regions, a right hydrothorax and streaky linear shadows in the right, upper lung field. Similar shadows were observed in the right, lower lung field following thoracentesis. No lesions were noted in complete skeletal x-rays. A barium enema revealed a normal colon and terminal ileum but showed the liver at the level of the right iliac crest and the spleen 4 cm. above the left iliac crest. Six hundred ml. of brownish yellow, semi-opaque pleural fluid with a specific gravity of 1.017 were removed by thoracentesis. The fluid contained 3,300 red blood cells and 242 white cells with 15 per cent neutrophils, 32 per cent eosinophils and 53 per cent lymphocytes per ml. Cell block of this fluid revealed no tumor cells or organisms. Bacterial smears and cultures of the nose, throat, sputum and pleural fluid were negative for pathogens. Guinea pigs inoculated with sputum and pleural fluid developed no gross or microscopic lesions. No ova, parasites or pathogenic bacteria were found in the stools and no plasmodia were seen in blood smears. Skin tests with a 1:500 dilution of tuberculin, and trichinella antigen were negative, as were serum agglutination tests with Brucella abortus and melitensis, B. typhosus and B. paratyphosus A, B and C.

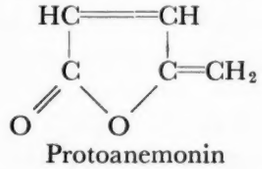
In view of the clinical picture and the patient's four years' residence in endemic areas of coccidioidomycotic infection, an intensive effort was made to demonstrate this fungus. Coccidioides immitis was isolated by Dr. R. W. Benham from blood cultures taken on January 20th and February 1st and 3rd. No organisms could be cultured from sputum or pleural fluid. Two coccidioidin skin tests in dilution of 1:100 were negative. The serum precipitin test was negative and the complement-fixation test was positive in a dilution of 1:32. Dr. C. E. Smith of Stanford University, who kindly performed the serologic tests, interpreted them as diagnostic of disseminated coccidioidomycosis.[29] The microscopic slide of the nasolabial skin biopsy taken six months previously was obtained at this time and C. immitis could be demonstrated at the center of the granulomatous lesions. (Fig. 1.) Not until some time later could the slides containing the uterine curettings of 1943 be obtained. When examined they, too, revealed C. immitis within the giant cells of the granulomatous lesions of the endometrium. (Fig. 2.)

Isolation of C. immitis from the patient's blood stream confirmed the diagnosis of disseminated coccidioidomycosis. This was amply supported by identification of the organisms within the lesions of the skin and endometrium and by the serologic tests. The negative coccidioidin skin tests were interpreted as due to overwhelming infection.

The course of the patient following admission was progressively downhill. Her temperature rose daily to between 103.6° to 106°F., her heart rate ranged from 92 to 172, averaging 140, and her respiratory rate varied from 30 to 60. Dyspnea and cyanosis were marked and unrelieved by oxygen, bronchodilators and digital-

ization. The patient developed alternating abdominal distention and diarrhea, deep pains in the left radius and both wrists, and peripheral edema appeared terminally. Five blood transfusions, each of 500 ml., were administered. Following the first two, generalized urticaria and facial edema appeared. To alleviate this and to prevent further reactions pyribenzamine was used with good results. Coincident with this medication the eosinophilia decreased nearly ten-fold, with the absolute eosinophil count falling from, 4,400 to 470.

The organisms isolated from the patient were tested *in vitro* by Dr. Beatrice C. Seegal against several antibiotics. The fungus grew well in media with added sulfadiazine, penicillin or streptomycin, but was inhibited by 5 mcg. per ml. of protoanemonin (a dilution of 1:250,000). Complete inhibition was obtained in a 1:125,000 dilution. Protoanemonin, $C_5H_4O_2$, is an antibiotic which is extracted from some species of the plant Ranunculaceae and has been found to possess *in vitro* activity against several fungi and bacteria.[26,27,28] Furthermore, the patient's serum did not inactivate the coccidioidostatic activity of protoanemonin *in vitro*.



Protoanemonin

Because of the terminal nature of the patient's condition and the lack of any known coccidioidocidal agent, it was decided to administer protoanemonin in view of its effect on the patient's organisms as demonstrated *in vitro*. The initial daily dose was to be as large as had previously been given to humans, with increase gradually to the point of tolerance. Protoanemonin dissolved either in 5 per cent glucose and water or in physiologic saline was given intravenously during the last six days of life. The respective daily doses were 135, 250, 500, 750, 1,000 and 1,150 mg. for a total of 3,785 mg. No beneficial effect of the drug was noted, either with regard to sterilizing the blood stream (as blood cultures taken on the fourth and sixth day of treatment grew out C. immitis) or with regard to the symptoms and signs. No signs definitely due to drug toxicity were observed; however, the hyperpyrexia, associated with frequent episodes of delirium and coma terminally precluded adequate appraisal of symptoms. The patient died February 3, 1946, the sixteenth day after admission. Permission for postmortem examination was refused.

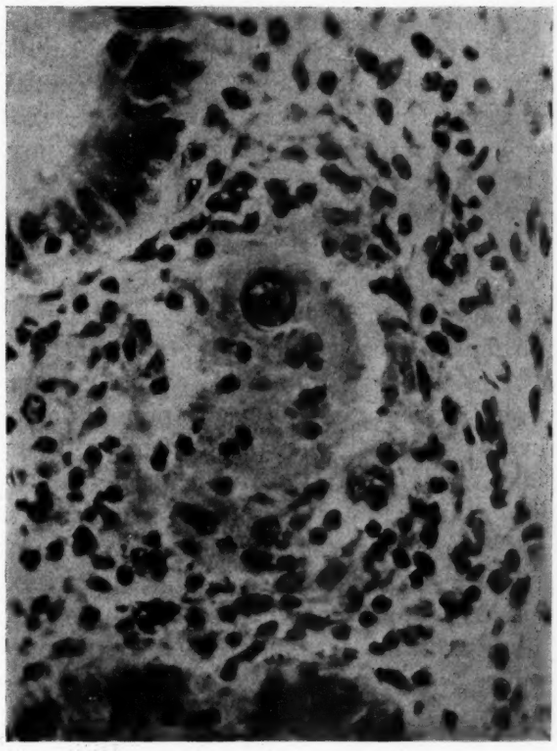


FIG. 2. Endometrial curetting, two months postpartum, taken on September 17, 1943, and revealing the doubly refractile spherule of Coccidioides immitis centrally placed in a granulomatous lesion of the endometrium.

COMMENTS

The case reported above is unusual in several respects. The history of this patient, supported by the endometrial biopsy, suggests that the disease may have began in the uterus during the puerperium. Previous pulmonary infection in her case is not likely in the absence of respiratory symptoms and in the presence of a normal chest x-ray at the time of the positive curettings. It seems improbable that normal conception, gestation and delivery would have been accomplished in the presence of coccidioidal endometritis. It is therefore conceivable that a primary coccidioidal endometrial infection may have occurred in this postpartum patient. There is no other example of this in the literature. However, two cases of widespread, symptomatic coccidioidomycosis of the internal female genital organs

have been reported. The first case, reported by Jacobson in 1929[24] which showed simultaneous pulmonary abnormalities by x-ray film, was apparently cured after a right oophorectomy and bilateral salpingectomy. The second case, interpreted as disseminated coccidioidomycosis, was also apparently successfully treated by a subtotal hysterectomy, left oophorectomy and bilateral salpingectomy.[25] Both of these cases drained purulent material containing C. immitis for a short time postoperatively. These cases probably represent hematogenous involvement of the Fallopian tubes with secondary tubo-ovarian abscess and endometritis as demonstrated by the microscopic findings. The involvement in these cases does not resemble that of the patient described herein as her infection was asymptomatic (the curettage was performed because of failure of uterine involution after two months); her gynecologic infection was probably limited to the endometrium and was apparently cured by a simple dilatation and curettage, following which regular menses reappeared. Four maternal deaths from disseminated coccidioidomycosis appearing during pregnancy have been reported recently,[32] with no indication that the primary focus was uterine.

The performance of the curettage may have induced dissemination which appeared clinically three months later when her two-year febrile illness began. The main objection to the suggestion of primary uterine infection in this case is that a pulmonary lesion may have existed in a quiescent state prior to the puerperium. However, this would presuppose endometrial involvement by hematogenous dissemination, which is unlikely here in the absence of salpingitis.

Protoanemonin was exhibited in this case because of the *in vitro* effectiveness against this patient's strain of C. immitis and because no other proven therapeutic agent is known. Occasional favorable reports[30,31] appear in the literature concerning the use of a vaccine prepared from a ball-mill grind of the fungus. Whether the *in vitro* coccidioidostatic activity of protoanemonin may be translated into *in vivo* usefulness cannot be determined from this case. The drug was used in a terminal and fulminating phase of disease but, none the less, blood taken after the last dose on the day of death grew out C. immitis. Furthermore it is not known whether the drug will act at all *in vivo* and, if it does, what concentration is required in body fluids containing the fungus.[26,27,28] Very small dosage was used in this case initially and was increased rather cautiously. All attempts to measure the concentrations of protoanemonin in the blood and urine of this patient were unsuccessful. However, it is suggested that the drug receive additional trial under more favorable conditions.

SUMMARY

A young white woman, while in an endemic area for C. immitis, conceived and delivered a normal baby who has remained healthy. Two months later a granulomatous but asymptomatic coccidioidal endometritis was discovered without a preceding history of respiratory infection or cutaneous lesion, at which time x-ray examination of the chest was normal. Three months later symptoms of generalized disease developed. Pulmonary lesions did not appear until nearly two years after the establishment of uterine infection. During this time repeated coccidioidin skin tests were negative, but a facial cutaneous coccidioidal granuloma appeared, followed by marked eosinophilia. Two and one-half years from its onset the disease became fulminating and terminated in death despite the use of protoanemonin which *in vitro* prevented the growth of the fungus isolated from the patient's blood. It is suggested that the uterus may have been the portal of entry of infection in this patient.

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Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria*

(*Marchiafava-Micheli Syndrome*)

N. Henry Simpson, Jr., M.D. *and* Ellis C. Oldham, M.S.

Little Rock, Arkansas *Des Moines, Iowa*

Chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria was first differentiated from other hemolytic anemias by Marchiafava and Nazari[17] in 1911. These observers reported three cases of hemolytic anemia and attention was directed to the "perpetual" hemosiderinuria in one of their cases. At autopsy an unusual distribution of iron pigment was found in this case. Fifty-two cases have been reported in the available literature. The consensus expressed in the literature is that splenectomy is unsuccessful in treatment of this condition. However, Dacie, Israels and Wilkinson[3] reported a case which apparently was benefited by splenectomy. It is the purpose of this paper to present a brief review of the literature, present a case giving the results of splenectomy and a few experimental observations.

CLINICAL DESCRIPTION

A salient feature of the disease is an anemia that is due to intravascular hemolysis.[8] The hemolysis is a continuous process, not associated with physical exertion or temperature change but is accelerated during sleep.[27] Remissions and exacerbations of anemia occur and are manifested by varying severity of hemolysis. Another striking feature noted during exacerbation is the red-black urine of the first morning specimen. The hemoglobinuria may be seen in only one morning specimen or it may persist. It usually clears as the day progresses. Chills, fever and malaise sometime accompany the hemolytic paroxysms.[10] Regardless of the hemoglobinuria a persistent hemosiderinuria can readily be detected in the sediment.[16]

The onset may be gradual or abrupt. The disease usually occurs in the third or fourth decades although younger and older age groups have been reported.[19,21] If the onset is gradual, symptoms of anemia are experienced before gross hemoglobinuria ensues. No sex predilection or hereditary tendencies have been reported.[21]

Physical findings include pallor of the skin and mucous membranes and a mild icterus. A slight to moderate enlargement of the liver and spleen frequently occurs.

Laboratory studies reveal an average anemia of 2 to 4 million erythrocytes with free hemoglobin values above 5 mg. per cent in the plasma or serum.† The plasma bilirubin is increased especially after bouts of excessive hemolysis.[20] A reticulocytosis as high as 40 per cent is present. The bone marrow shows increased erythropoietic activity. The erythrocyte fragility is normal and the Donath-Landsteiner test is negative. As first observed by Van den Bergh[26] and later investigated by Ham,[8] an increased susceptibility of erythrocytes to hemolysis is observed in an acid medium. When the patient's erythrocytes are added *in vitro* to acidified normal or patient serum, marked hemolysis results. The serum is acidified by adding sufficient amounts of mineral acids

† May be quantitated by the method of Bing and Baker.[1]

* From the Department of Internal Medicine, Veterans Administration Hospital, Des Moines, Iowa.

or equilibration with carbon dioxide to adjust the pH to 6.8 or 7.0. Usually a leukopenia of 2,000 to 4,000 showing a relative lymphocytosis is present. In Pierce and Aldrich's report of the disease in a child a higher count was recorded.[19] A thrombocytopenia of 100,000 to 150,000 with concomitant hemorrhagic tendencies has been observed in some cases.

The hemoglobinuria can be demonstrated spectroscopically or by means of the benzidine test on a centrifuged specimen. The urine sediment shows hemosiderin within the desquamated epithelial cells. This can be elicited by the addition of potassium ferricyanide to the sediment and observing it microscopically for blue particles within the epithelial cells.

Differential Diagnoses. Among the diagnoses to be considered are paroxysmal cold hemoglobinuria, favism, march hemoglobinuria, black water fever, congenital hemolytic icterus and toxic hemoglobinuria. In paroxysmal cold hemoglobinuria a correlation of chilling and hemoglobinuria is frequently obtained in the history. The serologic test for syphilis is usually positive and cold hemolysins can be demonstrated in the blood. Favism, common in Sicily, Sardinia and other parts of Italy, is caused by ingestion of fava beans (Vicia fava) or inhalation of its pollen. The disease has been reported in the United States.[13] March hemoglobinuria[7] occurs primarily in young, healthy adults and is precipitated by walking or running but is rarely severe enough to cause anemia. Black water fever is associated with chronic malaria of the falciparum or vivax type usually while the patient is undergoing antimalarial therapy.[5] A familial history of anemia may be obtained in congenital hemolytic icterus. Splenomegaly, spheroidocytosis and increased fragility are characteristic. The toxic hemoglobinemias[14] must be considered. In such cases there is usually a history of ingestion or exposure to toxic agents. Arsine poisoning, however, may be contracted from obscure sources, namely, chemical laboratories, submarines and industries "pickling" steel in acid.[18] The hemolytic effect of Clostridium welchii infection is well known.

Anatomic Findings. The morbid anatomy has been summarized by Scott et al.[21] The main features are: (a) venous thrombosis in the systemic and portal circulation; (b) hepatomegaly with central zonal necrosis; (c) moderate splenomegaly with little histological deviation from normal; (d) enlarged "snuff-brown" kidneys showing cloudy swelling; (e) hemosiderosis in the renal convoluted tubules and ascending loops of Henle. There are only traces of iron in other organs; (f) marked orthoplastic erythropoiesis of the bone marrow. The only feature constantly found is siderosis of the renal tubules.

Clinico-pathologic Correlation. A decreased amount of iron in the liver and spleen and increased quantity in the kidneys is to be expected considering the drainage of iron from the usual reservoirs and its subsequent excretion by the kidneys. The process of reabsorption of the hemoglobin by the tubules results in siderosis of the tubular epithelium. Desquamation of this epithelium accounts for the hemosiderinuria found even during the remission phases. Scott et al.[21] on the basis ot experiments in animals by Hjarre believe the central zonal hepatic necrosis is largely due to the mechanical effect produced by blockage of the liver sinusoids by agglutinated erythrocytes and erythrocytic stroma. Such particles are believed to be derived from intravascular hemolysis in the portal system. An analogous situation occurs in the systemic veins resulting in pulmonary emboli, thrombi in pulmonary capillaries and meningeal veins.

Pathologic Physiology. The extensive work of Ham and Dingle[9] who investigated the mechanism of hemolysis revealed that the fundamental abnormality resides in the erythrocytes. The abnormality has not been definitely defined. A serum factor present in the patient's or normal serum is essential for the hemolysis. The factor is present in the complement fraction. The thermolabile

portion of the factor cannot be restored by the addition of guinea pig or other animal sera but a thermostabile portion destroyed by zymin or ammonium hydroxide is restored in part by the addition of guinea pig serum. A hemolytic antibody either within the erythrocyte or in the serum could not be elicited by the methods employed. No increased susceptibility of erythrocytes to hemolysis in non-immunologic systems such as saponin or sodium taurocholate could be demonstrated. Ham and Dingle concluded that the hemolysis probably is due to abnormal erythrocytes which are hemolyzed in the presence of human complement. The hemolysis varies with the susceptibility of the erythrocytes to lysis as well as with the acidity of the serum. Ham postulated that with an increase in the carbon dioxide content of the blood during sleep a slight decrease of blood pH results, hence hemolysis follows. A decreased pH within physiologic limits was recorded in Ham's cases. Hoffman and Kracke,[12] however, failed to note this nocturnal decrease.

Therapy. Treatment is unsatisfactory. Liver and iron are of no apparent benefit. The administration of antacids such as sodium bicarbonate produces no lasting beneficial effect and even aggravates the hemolysis when given for a prolonged period.[8] Hemolysis is also increased following discontinuance of this therapy. Sympathomimetic and vagomimetic drugs, such as adrenalin, prostigmine, eserine sulfate and pilocarpine have been given.[12] The severity of the nocturnal exacerbations was lessened by adrenalin but parasympathetic stimulants were more efficacious. The anemia and reticulocytosis were not appreciably altered, however, by these substances. The administration of adrenalin and desoxycorticosterone was not effective. Blood transfusions given at proper intervals are most satisfactory even though a temporary increase in hemolysis may result. The hemolytic episodes are followed by periods of remission of hemoglobinuria and a subsequent increase in the erythrocyte count. Dacie and Firth[2] observed that transfused donor cells had a normal survival time and that the posttransfusion hemolysis is due to destruction of the patient's erythrocytes. The intravenous administration of normal, stored serum instead of whole blood had no appreciable effect upon the hemolysis. Splenectomy is generally considered of no value[8,10,20] and fatal results have been observed. These patients tolerate surgical procedures poorly.

CASE REPORT

M. J. O'B., a thirty-six year old male, of Irish descent, employed as hospital attendant, was admitted to the Des Moines Veterans Hospital, October 1, 1947, complaining of weakness and dyspnea on exertion of six months' duration. He had been in good health until March, 1947, when he had an upper respiratory infection accompanied by chills and fever lasting ten days. He was treated by his private physician with sulfonamide and sodium bicarbonate with an adequate fluid intake. On return to work he noticed weakness, easy fatigability and tachycardia on slight exertion. In August, 1947, he again had an upper respiratory infection accompanied by chills and fever. He was hospitalized and received intramuscular penicillin five days for treatment of "impending pneumonia." At this time he first noted "coffee-colored" urine in a noon specimen. The patient was told that he had an anemia of 2 million. He was given iron and parenteral liver therapy. Following discharge from the hospital he continued to take iron medication but noted paroxysms of "coffee-colored" urine lasting two to three days and occurring approximately every two or three weeks. His symptoms of weakness, fatigue and dyspnea increased and necessitated further hospitalization.

Physical examination revealed a well developed, well nourished, white male, 5 feet 10 inches tall, weighing 200 pounds, with a definite pallor. The sclerae had a yellowish tinge and the mucous membranes were pale. The liver was palpable about 2 cm. below the right costal border but the spleen was not. A linear scar on the left thigh extended from the left anterior superior iliac spine to the medial condyle of the left femur.

Roentgen studies revealed a normal chest plate; an intravenous pyelogram was made and colon and gastrointestinal series carried out.

These studies showed no abnormalities. The spleen could not be visualized in the left upper quadrant roentgenographically. A gastroscopic examination was negative. Gastric analysis showed no free hydrochloric acid in the fasting specimen but there were 38 degrees after histamine.

Precautions were taken to prevent hemolysis during the process of drawing and determining the plasma hemoglobin. All control samples were obtained by exactly the same process. Grossly, the patient's plasma or serum was always pink or red-brown. The plasma hemoglobin usually varied from 20 to 30 mg. per cent but during paroxysms ranged up to 414 mg. per cent. The controls showed less than 5 mg. per cent. The serum hemoglobin was slightly higher than that of plasma. Spectroscopically this was oxyhemoglobin and methemoglobin.

A representative erythrocyte count was 2.65 million; hemoglobin 8.0 gm. and hematocrit 29 cc./100 cc., giving a mean corpuscular volume of 109 cu. microns, mean corpuscular hemoglobin 30 micromicrograms and a mean corpuscular hemoglobin concentration of 28 per cent. Anisocytosis and polychromatophilia of moderate degree were noted on the stained smear. The white count most frequently ranged between nine and ten thousand with a differential as follows: neutrophiles 61 per cent, lymphocytes 36 per cent, monocytes 1 per cent and eosinophiles 2 per cent. Counts higher and lower than the above were obtained and the differential occasionally showed a predominance of lymphocytes. The reticulocyte count varied up to 13.5 per cent and the thrombocyte value ranged between 300,000 and 400,000. An increased degree of hemolysis of the patient's washed erythrocytes was obtained when these were suspended in acidified normal or patient's serum, as advocated by Ham. Erythrocyte fragility test showed beginning hemolysis at 0.48 per cent and complete hemolysis at 0.32 per cent. The Donath-Landsteiner and Kahn tests were negative. Auto- and isohemolysins as well as auto- and isoagglutinins could not be demonstrated by the method of Dameshek and Neber.[4] Clot retraction was complete after four hours. The bleeding time was two minutes, coagulation time four minutes. The sedimentation rate was 40 mm. in sixty minutes by Cutter's method. The blood urea nitrogen was 12.5 mg. per cent; sodium chloride was 476 mg. per cent; uric acid 3.2 mg. per cent; carbon dioxide combining power during sleep, 44 volumes per cent; CO_2 combining power in morning, awake, was 51 volumes per cent. Total serum proteins 5.6 gm. per cent. The albumin was 3.8 and globulin was 1.8 gm. per cent.

A hyperplasia of erythrocytic and granulocytic elements with many blast cells and frequent mitotic figures occurred in the sternal marrow smears.

The serum bilirubin varied from 0.9 to 6.0 mg. per cent. Urine urobilinogen was 0.14 mg. per cent in 114 cc. urine, giving 0.66 Ehrlich units (when serum bilirubin was 0.9). The direct Van den Bergh was positive. Thymol turbidity was 10.5 units with a cephalin-cholesterol flocculation value of 1 plus. The intravenous bromsulfalein test (5 mg./kg. body weight) showed 62 per cent retention after five minutes and seven per cent after forty-five minutes. The oral hippuric acid test showed 3.5 gm. excreted, and the prothrombin time by Quick's technic varied between 64 to 90 per cent of normal.

The urine showed albumin in amounts varying from a trace to 4 plus. The maximum specific gravity was 1.025. The minimum concentration was 1.005. Microscopically, occasional coarsely and finely granular casts were present. An average of two to six leukocytes and occasional erythrocytes were seen in many urine specimens. There were instances when no erythrocytes were seen in a fresh specimen even though hemoglobin was present. During paroxysms of hemolysis fractional urine specimens showed hemoglobin in concentrations as follow: First voided specimen, after arising, 990 mg. per cent; from 8:00 A.M. to 12:00 N., 194 mg. per cent; from 12:00 N. to 4:00 P.M., none; and from 4:00 P.M. to 10:00 P.M., none. On one occasion the morning urine showed a hemoglobin concentration of 2500 mg. per cent. After adding potassium ferricyanide to the urine sediment, blue intracellular particles could be demonstrated microscopically within the epithelial cells. This was found even during times when the urine was negative for hemoglobin. The standard urea clearance value was 92 per cent of normal. The intramuscular PSP test is as follows: After one hour, 15 per cent dye was excreted; after two hours a total of 30 per cent.

The stools gave negative reactions to benzidine when the patient was on a hemoglobin-free diet.

The patient was given liver and iron as supportive measures but this had no effect upon

the anemia. In a period of nine month's hospitalization he received 33 pints of whole blood. The transfusions were usually followed by paroxysms of hemoglobinuria but at no time was there any evidence of thromboses. On recommendation of the hematology consultant and chief of surgery the patient underwent a splenectomy June 25, 1948. He received three 500 cc. transfusions of whole blood on June 24, 1948, and four on the day of surgery. Eight hours after surgery the patient had hemoglobinuria but for the remainder of the hospital period no further episodes occurred and the patient had an uncomplicated recovery from the operation. The blood counts were as follows:

Date	6/24	6/25	6/26	6/27	6/28	6/30	7/3	7/5	7/6	7/29	8/12	9/20
Million R.B.C.	3.78	.....	4.39	4.94	5.40	5.18	5.00	5.00	5.06	4.34	4.31	2.10
Hb.	11.	14.5	14.	15.	15.5	15.	14.5	14.5	14.5	12.5	11.5	9.5
Hematocrit	39	.....	.....	.....	.....	49	.....	.....	.....	.....	40	.....
W.B.C.	10,500	12,000	16,500	18,600	18,900	15,200	15,200	9,700	8,200	11,200	18,000	12,400
Neutrophils	76	.....	88	91	78	74	79	71	63	53	83	41
Lymphocytes	24	.....	11	8	21	23	19	28	36	41	9	49
Monocytes	.....	.....	0	.....	1	2	1	.....	.....	3	.....	3
Eosinophiles	.....	.....	1	.....	.....	1	1	1	1	3	3	.....
Basophiles	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	2*	4
Platelets thousands	.....	393	460	583	510	401	500	810	550	350	.....	370
Normoblast	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.4%

* Disintegrated cells, 3.

The spleen was of usual shape and contour and weighed 250 gm. An accessory spleen 0.8 cm. in diameter was present. The consistency was soft and the surface made by section was smooth, glistening and protruded slightly from the surrounding capsule. The report on the microscopic section was: "Section of the spleen reveals an intact capsule and prominent trabecular markings. Lymphoid follicles are numerous and appear hyperplastic with large germinal center surrounded by dense collections of mature lymphocytes. The venous sinusoids are markedly congested by erythrocytes in varying stages of hemolysis. Cellular detail of the white pulp is indistinct. The reticulo-endothelial elements of the stroma appear hyperplastic and phagocytes containing brown pigment in their cytoplasm are numerous. Many areas of recent hemorrhage are observed in the substance of the splenic tissue. Numerous small arterioles with thickened walls are also apparent."

Liver biopsy revealed no focal lesions. The microscopic report was as follows: "The sections of liver display a thin capsule and pale staining cords of liver cells. The cell boundaries are indistinct and there is a vacuolated or vesicular appearance to the cytoplasm. Many of the liver cells contain granules of brownish pigment. Sinusoids near the capsule are congested with erythrocytes and a mild bile duct hyperplasia is apparent. No areas of necrosis or neoplasia are seen."

The anatomic diagnoses were: (1) spleen, showing congestion; (2) accessory spleen.

About six weeks after splenectomy the erythrocytes still showed an increased susceptibility to acids but no free hemoglobin was found in the serum. The bone marrow still revealed a hyperplasia of all elements. The reticulocyte count was 0.6 per cent. The serum bilirubin was 0.7 mg. per cent; the urine urobilinogen was 0.20 mg. in 186 cc., and the thymol turbidity was 7 units. There was no hemosiderin or hemoglobin in the urine. A postoperative phenolsulfonphthalein test showed 93 per cent excretion after two hours. The non-protein nitrogen was 32 mg. per cent. Subjectively the patient felt much stronger, had a return of libido and experienced no dyspnea. He was given a leave of absence but reported briefly for check-up on September 20, 1948, stating that he had noticed two paroxysms of hemoglobinuria about ten weeks after operation but subjectively had no symptoms of anemia.

Physical examination at his return revealed pale mucous membranes and an icteric tint of the skin. The splenectomy incision was well healed and the liver was just palpable. His count at the time of the check-up was as indicated on September 20, 1948. The acid hemolysis test was positive. Free hemoglobin

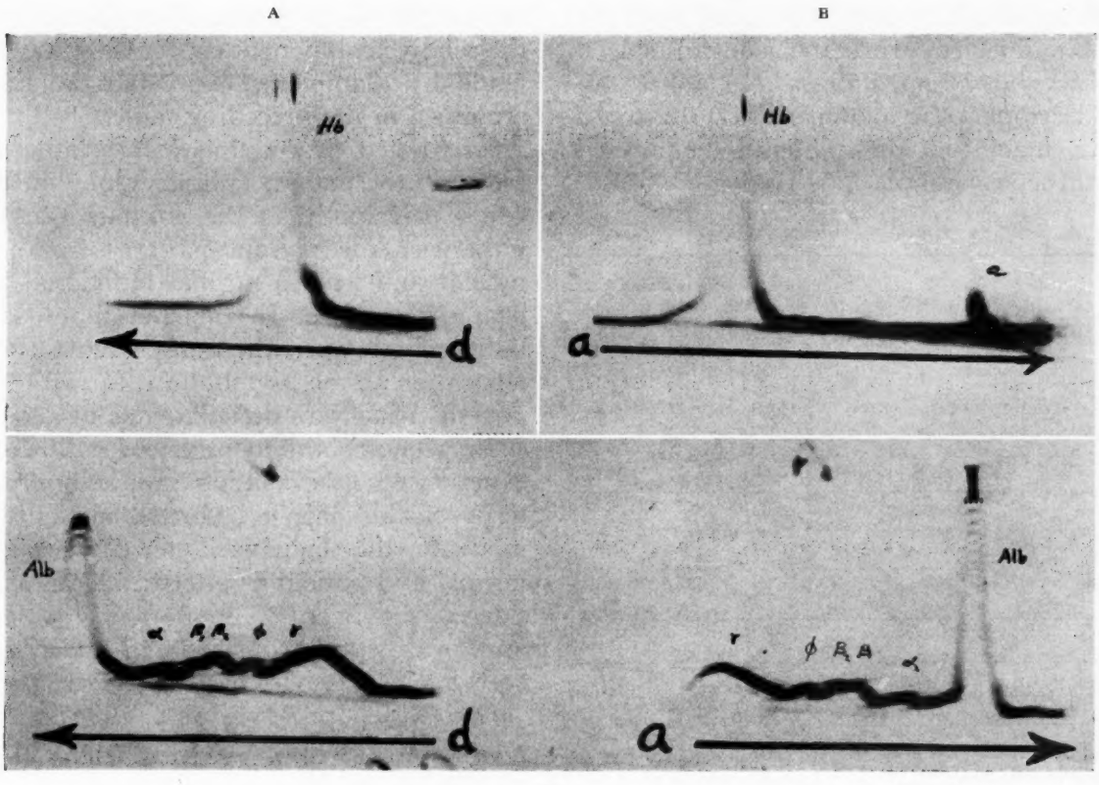

FIG. 1. A and B, electrophoretic diagram of erythrocyte hemolysate from the case of paroxysmal nocturnal hemoglobinuria presented. C, electrophoretic diagram of plasma from case presented.

(oxyhemoglobin and methemoglobin) was demonstrated in the serum. A large amount of hemosiderin was found in the urine. He was given transfusions and discharged.

Since then he has returned to the hospital with symptoms of anemia. Treatment has consisted of multiple transfusions. Liver and kidney function tests have shown progressive impairment. During his most recent hospitalization he was given pilocarpine 3 mg. subcutaneously twice daily at 3:00 A.M. and 3:00 P.M. for seven days. While receiving pilocarpine the urine showed a trace of hemoglobin on one occasion. The severity of anemia was not considered to be significantly altered by this therapy.

EXPERIMENTAL

Considering the possibility that an erythrocyte-sensitizing substance might be present in the patient's spleen, similar to the suggested thrombocytolytic agent "thrombocytopen,"[11,23–25] the following investigation was made. The spleen as received from surgery was promptly sectioned and frozen in dry ice. Two 20 gm. portions were ground in a Waring blendor with 30 cc. normal saline and extracted twenty-four hours with occasional shaking in the refrigerator. About 15 cc. of extract were filtered under suction, divided into 1 cc. portions and immediately frozen and stored at −5°C. A duplicate control extract was prepared from an autopsy spleen.

Daily 1 ml. intravenous injections of the extracts were made into the marginal ear vein of three 2-kg. rabbits over a period of eleven days. Two of the rabbits were injected with extracts from the patient's spleen and the remaining rabbit was injected with the extract from the autopsy spleen. Complete blood counts taken on the rabbits injected with patient's spleen extract before and immediately following the injections showed no significant change. Likewise, acid hemolysis tests on the rabbit erythrocytes before and after injection showed no increase in sensitivity to serum acidified

with hydrochloric acid. The blood from the rabbit injected with the control spleen extract showed no changes.

Electrophoretic patterns (Fig. 1A to C) were made on the patient's hemolyzed erythrocytes and plasma. The object of this

ERYTHROCYTE HEMOLYSATE

		Mobilities ($\times 10^{-5}$ cm.^{2} per volt per sec.)		Relative Per cent Composition	
		a Protein[28]	Hemoglobin	a Protein	Hemoglobin
Patient's erythrocytes	Ascending	6.89	2.67	3.2	96.8
	Descending	*	2.62	*	*
Normal erythrocytes	Ascending	5.69	1.89	5.3	94.7
	Descending	6.02	1.68	4.6	95.4

PLASMA

Patient's plasma	Albumin	α	β_1	β_2	ϕ	γ
Mobilities $\times 10^{-5}$	6.1	4.6	3.9	3.4	2 8	1.5
Relative per cent composition	51.5	4.9	5.2	6.4	5.3	26.7
Normal plasma mobilities $\times 10^{-5}$	6.0	4.5	3.9	3.4	2.8	1.6
Relative per cent composition	57.4	6.1	5.7	7.3	4.3	19.3

* Not determined.

experiment was to demonstrate if possible an abnormal red blood cell pattern.* The packed red cells were washed repeatedly and hemolyzed by the dry ice-acetone freezing method used by Stern, Reiner and Silber.[22] The sample for electrophoresis was prepared by dilution with nine volumes of a phosphate-NaCl buffer before dialysis. The phosphate buffer had a pH of 7.56. Sodium chloride was added to bring the ionic strength to 0.18. Electrophoresis was accomplished in a modified Klett apparatus at 140 volts and 40 milliamperes. Durations of run were 14,800 seconds to 16,500 seconds. A twelve-hour period of standing was allowed in some instances after completion of run before photographing the patterns. This was to allow for diffusion to reduce the height of the peaks. Measurements of the relative component concentrations were made by planimetry. The data obtained are given in the preceding table.

It is noted that there was a relative increase in the patient's plasma globulin, this being principally in the gamma fraction. Otherwise the plasma patterns from the patient and from a normal individual appeared the same. In the phosphate-NaCl buffer system used there appeared to be a separation of the β globulin into two components which are designated as β_1 and β_2.

In view of the considerable difficulty experienced in obtaining satisfactory patterns of the deeply colored red cell hemolysate, the slight variation between our normal and patient's patterns was not considered significant. The patterns did not appear different from those of Stern and co-workers. It has been suggested that variation in proteins absorbed on the erythrocytes could account for some difference in the pattern obtained.[7]

SUMMARY AND CONCLUSIONS

1. Detailed clinical and laboratory studies are reported in a case of chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria.

2. The clinical findings conformed in general to those previously described in available literature. The condition is manifested by chronic anemia due to intravascular hemolysis occurring at an increased rate during sleep. Physical examination showed icterus and mild enlargement of the liver but the spleen was not palpable.

3. A hyperchromic normocytic anemia was present and the plasma hemoglobin was consistently elevated. Hemosiderinuria and paroxysms of morning hemoglobinuria were noted. The acid hemolysis test of Ham was positive. Liver and kidney function tests showed impairment.

4. The patient underwent splenectomy but the transient benefit derived therefrom may best be attributed to multiple transfusions received immediately prior to and during surgery.

* Acknowledgement is made for the kind assistance of Dr. J. F. Foster, Dept. of Chemistry, Iowa State College, in making these electrophoretic runs possible.

5. Attempts to produce anemia and increased acid hemolysis of erythrocytes in rabbits by *in vivo* injections of saline extract from the patient's spleen were unsuccessful.

6. The electrophoresis patterns of the patient's plasma and hemolyzed erythrocytes are herein shown. Other than a slight increase in gamma globulin concentration there is no deviation from normal in the patient's plasma. The patterns obtained from electrophoresis studies on normal and patient's erythrocytes were not identical, although the small variations from normal are probably attributable to adsorbed plasma proteins on the patient's erythrocytes and difficulties in working with a highly colored solution.

The authors wish to express their appreciation to Drs. Daniel J. Glomset, Chief of Medical Service, K. R. Cross, Chief of Department of Pathology, and Louis T. Palumbo, Chief of Surgical Service, Veterans Administration Hospital, Des Moines, Iowa, for their cooperation and constructive criticism in the preparation of this paper.

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Shigella Alkalescens as a Cause of Pyelocystitis with Bacteremia

GEORGE C. SUTTON, M.D. *and* ARTHUR BERNSTEIN, M.D.
Chicago, Illinois

THE organism now known as Shigella alkalescens was first isolated by Andrewes[1] in 1918. He recovered the bacillus from the stools of patients convalescent from dysentery and did not consider it to be pathogenic for human beings. It was classified as an atypical dysentery bacillus resembling the Flexner type and was named because it gave a characteristic, strongly alkaline reaction in litmus milk. He observed "No case has been met with in which it (Shigella alkalescens) has been agglutinated by the serum of the patient from whom it has been isolated."

Since Foerster's observation in 1918[2] of pyelitis caused by the Flexner bacillus it has become recognized that shigella of both the Flexner and Hiss-Russel Y type are rather frequent causes of pyelocystitis.[3] However, proof that S. alkalescens is truly pathogenic for human beings has been only recently established.[4] A review of the literature to date reveals but twenty-three reported instances of recovery of S. alkalescens from the urine of individuals with clinical signs of pyelocystitis. (Table 1.) In only four of these cases has S. alkalescens been recovered from both the urine and blood.

In 1928 Smith and Fraser[5] reported the first occurrence of S. alkalescens bacteremia in a woman four days after an uncomplicated delivery; blood, urine, feces and uterine swabs all contained the organism. Her clinical course was chronic and she remained febrile for a month under nonspecific therapy. In the patient's serum agglutinins for the blood strain developed in a dilution of 1:120 and for the urinary strain in a dilution of 1:480.

The second case was reported by MacKenzie and Ratner.[8] This patient was a thirty-one year old woman with signs of right pyelitis. S. alkalescens was isolated from the urine, stool and blood. She was treated symptomatically and had a slow clinical response. No agglutination studies were made other than the Widal, which was negative.

Starkey[9] presented the case of a thirty-one year old woman who had a history of diarrhea and constipation a year previously. Acute pyelocystitis developed, with a pure culture of S. alkalescens in her urine and blood only. No agglutination reaction developed throughout the illness. However, the systemic reaction was demonstrated by precipitin and complement fixation tests. Slow improvement was achieved by treatment with urotropin.

In 1948 Cardon and Felsenfeld[19] published a case report of a twenty-two year old nurse with dysentery and pyelocystitis. S. alkalescens was identified in the blood and urine. The serum of the patient agglutinated the S. alkalescens strain isolated from the patient in a dilution of 1:320. Recovery was made rapid by treatment with sulfadiazine, and the organism was inhibited by 2 mg. per 100 cc. sulfadiazine *in vitro*. To these cases of S. alkalescens pyelocystitis with bacteremia is added one observed at the Cook County Hospital.

CASE REPORT

A nineteen year old married colored woman, a power machine operator, was admitted to the hospital on March 17, 1948, with a history of an aching pain in her right back for four days. The pain had increased in intensity and was accompanied with moderately increased fre-

quency of urination. She vomited once and felt feverish twenty-four hours before admission.

Physical examination revealed a well nourished young Negro woman who was toxic with a fever of 104°F., pulse, 120, regular rhythm and a respiratory rate, 28. Physical signs were entirely negative except for definite tenderness on palpation over the right kidney area. There was marked pain on percussion over the right costovertebral angle. A white, creamy, cervical discharge was noted and a Gram's stain of it showed many extracellular gram-negative cocci.

Admission urinalysis revealed many clumped white blood cells and a positive test for albumin. Urine and blood culture taken at that time produced a pure culture of S. alkalescens. A diagnosis of acute right pyelitis with cystitis was made and the patient was given sulfadiazine, 4 gm. initially and 1 gm. every four hours. Eight hours later her white blood cell count was 12,150 with 80 per cent polymorphonuclear cells, her red cell count 4,000,000 and hemoglobin of 68 per cent. The non-protein nitrogen was 32 mg. per cent, the total protein 7.4 gm. per cent and the Kahn test was negative. A flat plate of the abdomen revealed no pathologic disorder. The fever dropped rapidly and on the fifth day of therapy (March 22, 1948) the patient became afebrile and well and has remained so.

On the seventh hospital day both urine and blood cultures were sterile. The stool cultures contained no enteric pathogens. Four subse-

TABLE I

Year	Authors	Sex	Organism Present In:			Serum Agglutination Titer	Treatment and Response
			Stool	Urine	Blood		
1928	Smith and Fraser[5]	F	+	+	+	Blood strain 1:120 Urine strain 1:480	Not stated
1929	Weil, A. J.[6]	F	−	+	−	Not stated	Not stated
1931	Popoff and Spanswick[7]	F	+	+	0	1:640	Therapeutic abortion with good response
1934	MacKenzie and Ratner[8]	F	+	+	+	Negative Widal No agglutinins	Symptomatic, poor response
1934	Starkey[9]	F	0	+	+	Positive precipitin and complement fixation tests	Urotropin, poor response
1934	Murray and Pike[10]	−	0	+	0	1:640	Not stated
1936	Snyder, M. and Hanner, J.[11]	F	+	+	0	No agglutinins	Urotropin, poor response
1938	Neter, E.[12] Case 1	F	0	+	0	No agglutinins	Prontylin, rapid response
	Case 2	F	0	+	0	1:500	Mandelic acid, good response
1938	Neter and Rappole[13]						
	Case 1	F	0	+	0	Agglutinins to unstated titer	Not stated
	Case 2	F	0	+	0	Agglutinins to unstated titer	Not stated
	Case 3	F	0	+	0	No agglutinins	Not stated
1938	Wooley, P. V. and Sweet, M.[14] Case 1	F	+	+	0	Not stated	Ketogenic diet, responded
	Case 2	F	0	+	0	Not stated	Methenamine, responded
	Case 3	F	+	+	0	1:1280	Symptomatic, responded
	Case 4	F	0	+	0	No agglutinins	Methenamine and NH_4Cl, slow response
1938	Nabarro, D. and Edward, D.[15]	−	+	+	0	No agglutinins	Not stated
1943	Welch and Mickle[16] 4 cases	−	0	+	0	Not stated	Not stated
1943	Roux, P.[17]	−	+	+	0	Not stated	Not stated
1948	Cardon, L. and Felsenfeld, O.[18]	F	0	+	+	1:320	Sulfadiazine, rapid response

quent stool, urine and blood cultures showed similar results.

Tests of the patient's serum were carried out as follows: There was no agglutination with typhoid "H" and "O," paratyphoid "A" and "B" and Brucella antigens. However, on the fifteenth day of illness (March 28, 1948) the serum agglutinated the organism isolated from the patient's urine to a dilution of 1:1,280. On the twenty-fifth day of illness (April 7th) the agglutination titer for that organism was 1:640. By the thirty-ninth day of illness (April 21st) the serum agglutinated the urinary strain in a dilution of only 1:320; there was no agglutination of the organism isolated from the blood.

COMMENTS

The study of this case lends additional evidence that S. alkalescens is pathogenic for human beings. Review of the literature reveals that it is occasionally the cause of pyelocystitis and rarely the cause of bacteremia. Development of serum agglutinins of high titer during the acute phase of this case and their subsequent rapid decline is further evidence of the pathogenicity of this organism. In one other case[9] in which serum agglutinins were not demonstrable, precipitins and complement fixation reactions were detected.

It is worth while to note that Neter[12] in 1938 described a rapid recovery with the use of prontylin. A drug of the sulfonamide group was not used again until 1948 by Cardon and Felsenfeld.[18] All other cases were treated either symptomatically or with the commonly used urinary tract medications. Although no mortality has been reported, the morbidity has been decreased by the use of sulfonamides.

SUMMARY

1. All reported cases of urinary tract infection due to Shigella alkalescens have been summarized. Four cases of pyelocystitis with bacteremia have been analysed and an additional case reported.

4. There is a prompt therapeutic response to administration of drugs of the sulfonamide group.

Acknowledgment: We are indebted to Dr. Oscar Felsenfeld, M.D., head of the Department of Bacteriology, Cook County Hospital, for the bacteriologic and serologic studies.

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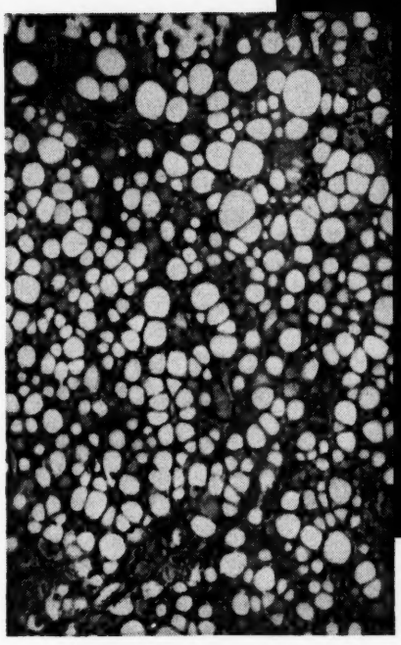

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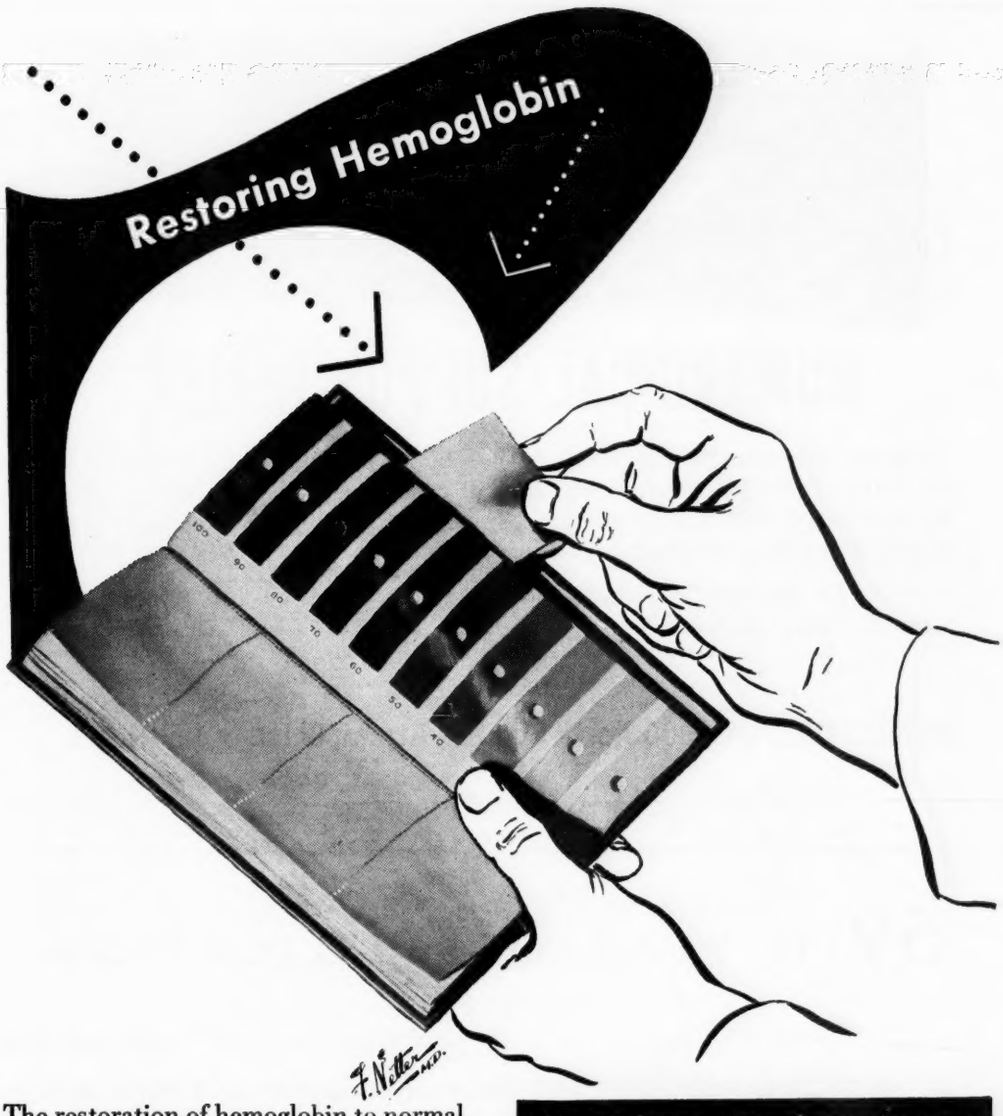

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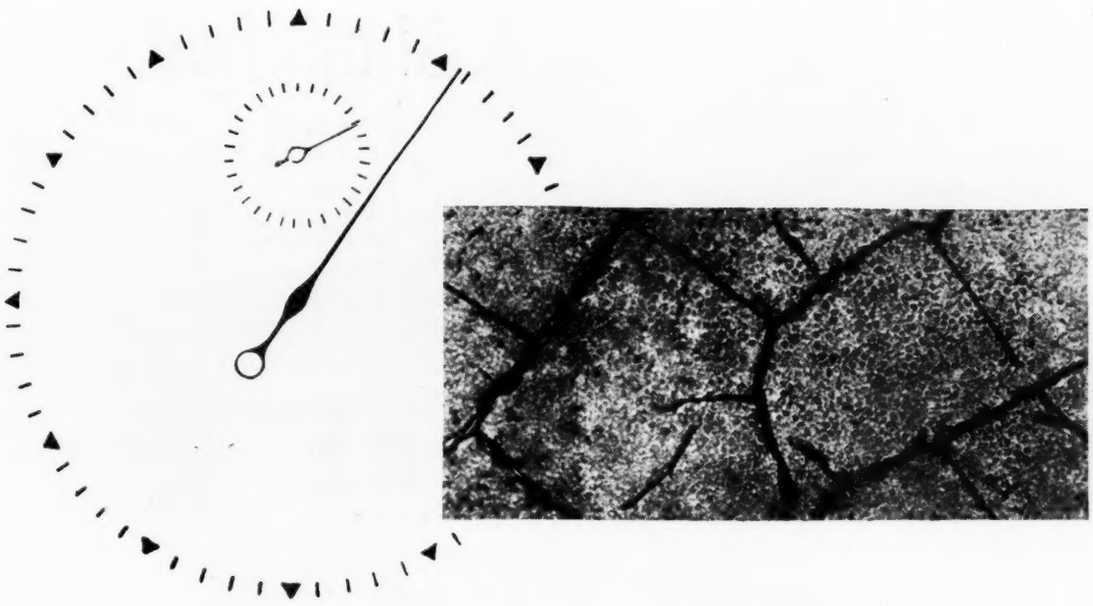

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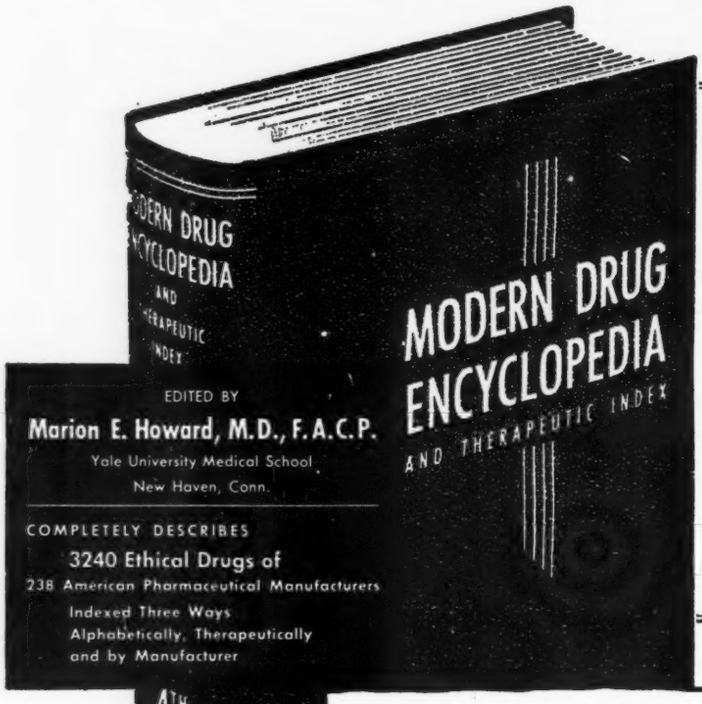

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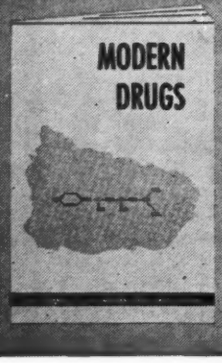

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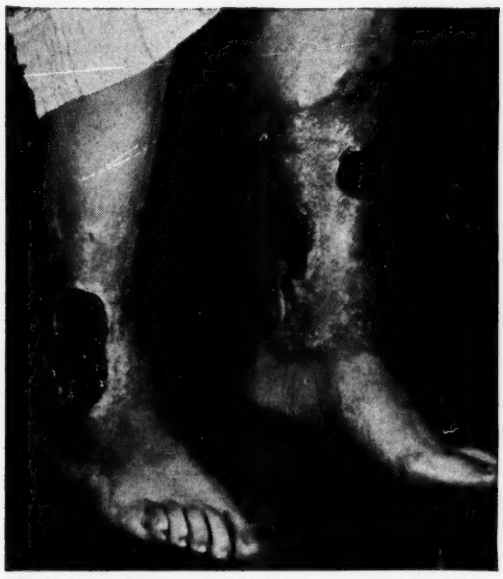

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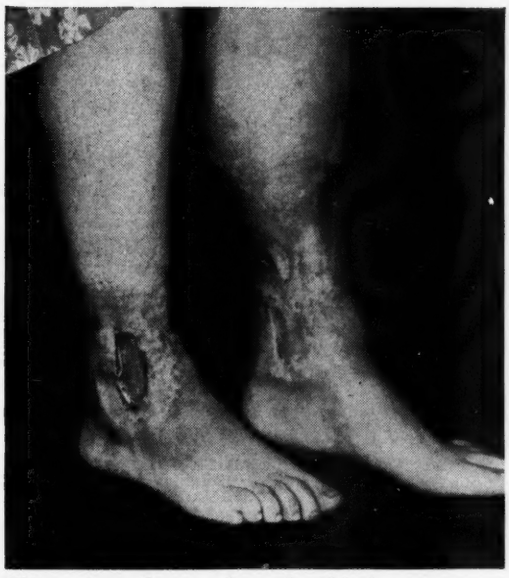

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***From the Lahey Clinic Bulletin** (Vol. 4, No. 8, April 1946): "The water-soluble chlorophyll containing ointment (*Chloresium*) has now been used at this clinic in more than 50 cases of the more chronic and difficult ulcers . . . (it) apparently excels any of the previously used agents . . . Many patients who had ulcers unhealed from one to eight years obtained complete healing in six to ten weeks."

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1. Ackerman, C. E.: N. Y. State J. of Med., 47:775, 1942.

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